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Effect of vitamin B12 and of animal proteins on the histopathology and hematology of chicks receiving an all-plant ration

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EFFECT OF VITAMIN B_{12} AND OF ANIMAL PROTEINS ON THE HISTOPATHOLOGY AND HEMATOLOGY OF CHICKS RECEIVING AN ALL-PLANT RATION

by

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I. INTRODUCTION

The story of the "animal protein factor" began approximately thirty years ago when pernicious anemia patients were treated with diets containing liver. Prior to this time, little was known about dietary deficiencies in relation to the anemias. As time passed on, scientific investigations revealed the presence of dietary factors in various foodstuffs which were necessary to obtain maximum growth of simple-stomached animals and of microbial species.

The numerous compounds interfering with the growth of various bacteria in producing a suitable microbiologic assay for the animal protein factor, more specifically vitamin B_{12}, have limited the progress in determining the vitamin B_{12} content of natural feeds. The need for digestion, at least partial digestion, of protein to render vitamin B_{12} available to these bacteria and to the protozoan, Euglena gracilis, has also been a limiting factor in establishing a specific microbiologic assay of natural feeds.

With these limitations in mind, macrobiologic assays have been developed for vitamin B_{12}. The rat, mouse, and chick assay have been described. One must realize the longer time required in making an assay by this method in

comparison with the microbiologic method. Therefore, it appears that more attention should be directed to the microbiologic method and then applied to the simple-stomached animals which require a dietary source of vitamin B_{12}.

Growth reponse has been the principal criterion in evaluating the vitamin B_{12} content of materials being assayed. It seems that other criteria should be checked along with growth response to aid in establishing adequate levels of vitamin B_{12} in rations to produce maximum growth. When maximum growth is obtained by the addition of vitamin B_{12} to rations low in the vitamin but adequate in other nutrients, it appears that such rations may become unbalanced. Thus, pathologic changes may be present in tissues. Also, one must keep hypervitaminosis in mind as this condition has occurred with other vitamins.

This experiment was designed primarily to compare the changes observed in the blood and other tissues of chicks in addition to growth response, by feeding different levels of vitamin B_{12}. One group received the basal ration whose protein was of all-plant origin, a second group received 3% liver meal in place of 3% of its soybean meal, and a third group received 0.5% APF concentrate in place of 0.5% soybean meal. The vitamin B_{12} activity of the APF concentrate was 12.5 mg. per pound as measured by the L.L.D. (Lactobacillus lactis Dorner) method of assay.

One hundred and twenty-six erythrocyte, leucocyte, and differential counts were made along with the same number of hemoglobin determinations, hematocrit readings, and sedimentation rates taken at one-half, one, two, three, and six hours. Statistical analyses of these data have been made. Sciatic nerves from 18 chicks (six from each group) were examined with the polarizing microscope. Various tissues from these 18 chicks were stained with hematoxylin and triosin. Corresponding tissues were also stained according to the Marchi method.

II. REVIEW OF LITERATURE

A. Vitamin B_{12}

1. Discovery, sources, and functions

Addison (1849) first described pernicious anemia. Elders (1925) suggested that pernicious anemia was a deficiency disease. He had cured sprue and pernicious anemia patients by dietary treatment since 1916. This treatment consisted of feeding patients daily 1 kg. of "undone" meat, 1.5 to 2 liters of raw milk, 15 cc. aqua calcis (lime water) five times a day, 15 or 20 cc. of cod liver oil, and a few oranges or 400 gm. of strawberries. Elders observed that hydrochloric acid was lacking in gastric juice from patients afflicted with pernicious anemia and sprue. It is known today that successful treatment of pernicious anemia corrects the achlorhydria.

Robscheit-Robbins and Whipple (1925) have conducted several experiments checking the efficacy of various foods in correcting anemia in dogs produced by bleeding. They found that beef liver produced maximum regeneration of hemoglobin and erythrocytes in severe anemia. Beef heart was less favorable than liver, and beef skeletal muscle

was less favorable than beef heart. The skeletal muscle was still considered to be a source of the factor or factors necessary for regeneration of hemoglobin and erythrocytes although it was not as potent as liver or heart.

Koessler et al. (1926) concluded that vitamin A was essential for blood regeneration. He was concerned with the treatment of pernicious anemia by improving the diet and administering vitamins A, D, and B (the vitamins known at that time). The diets which were recommended included liver, kidney, sweetbread, brain, or beefsteak. Perhaps the meat furnished the factor which stimulated blood regeneration instead of vitamin A. Minot and Murphy (1926) reported successful treatment of 45 pernicious anemia patients with a special diet. These patients received this diet from six weeks to two years. Three patients discontinued the diet and showed no improvement. The diet consisted of foods rich in iron, protein, and low in fat. It contained liver and an abundance of fruits and fresh vegetables. Before treatment the erythrocyte count averaged 1,470,000 per cu. mm. and after treatment for one month the count averaged 3,400,000. The average count for 27 cases observed four to six months after the diet was begun was 4,500,000 per cu. mm. of blood.

Minot and Murphy (1927) reported the findings of 105 cases of pernicious anemia treated from three months to

three years with mammalian liver. The general health and erythrocyte count improved in all patients receiving 200 grams of cooked liver daily. The erythrocyte count of 99 patients of 4,000,000 or more per cu. mm. was reached in two months when the diet was adequate. Some patients showed erythrocyte counts of 5,700,000 per cu. mm. Parkhurst (1927) revealed his findings based on four years of study with regard to normal hatchability of eggs. He found that animal protein was necessary in the breeding rations of hens in order to obtain eggs of high hatchability. This discovery was the first of its kind to associate animal protein with hatchability. Since Parkhurst's discovery, various feeds have been found to promote hatchability and chick growth. A review of some of this work will now be presented along with data on pernicious anemia and functions of vitamin B_{12}.

Byerly et al. (1933a) reported that hens fed a basal diet consisting of yellow corn, wheat bran, rolled oats, and alfalfa meal together with a mineral mixture and cod liver oil gave low egg production with fair hatchability. However, when 20% supplements of crab-scrap meal, buttermilk, or a combination of meat meal, fish meal, and buttermilk were added to the basal ration, production and hatchability were good. Twenty per cent supplements of North Atlantic fish meal and soybean meal gave good egg production

with low hatchability. With cottonseed meal, production was delayed and the hatchability was low. The results with yeast were not as good as with the basal ration alone. The same investigators (1933b) studied the effect of cereal products and animal proteins on the mortality of egg embryos. They found that cereal products supplemented with alfalfa leaf meal, minerals, and cod liver oil caused mortality of egg embryos during the second week of incubation. Byerly et al. (1933b) also reported that North Atlantic fish meal, crab meal, buttermilk, a combination of buttermilk, North Atlantic fish meal, and meat meal, and free range enabled hens to produce eggs whose embryos were normal.

Castle (1934) published an excellent review of his work dealing with the extrinsic and intrinsic factors as they are related to pernicious anemia. He believed that some physiological mechanism is not functioning properly in patients with pernicious anemia. The food (extrinsic factor) and stomach (intrinsic factor) are involved in this process. In his opinion pernicious anemia may be caused by a lack of intrinsic factor of the stomach, a lack of the extrinsic factor from food, or failure of absorption or destruction of these substances or their end product in the intestinal tract.

Van der Hoorn (1935) demonstrated that crude casein contains a growth-promoting factor for chicks. This factor

could be extracted with dilute acetic acid. Chicks fed purified casein instead of crude casein in the synthetic diet developed a leg deformity at three weeks of age. This condition was called "arthritis" and was not perosis. The tibial-tarsal joint was swollen and contained excess fluid. The growth-promoting factor and arthritis-preventing factor were removed during the purification of the casein. They were shown to be different factors. The arthritis-preventing factor was considered a mineral.

Nestler et al. (1936) reported that hens fed a basal ration consisting of 52.6% ground yellow corn, 25.8% wheat bran, 15.8% rolled oats and 5.8% alfalfa leaf meal received adequate vitamin G (riboflavin) to meet the minimum requirements for hatchability but lacked some other factor necessary for high hatchability. The unknown factor was present in dried pork liver, green grass, and to some extent in a mixture of desiccated meat meal, North American fish meal, and dried buttermilk. Dried whey was not a good source of the hatchability factor.

Greenspon (1936) stated that pepsin was antagonistic to the antipernicious anemia factor in the stomach (Castle's intrinsic factor). This work also disproves Castle's extrinsic factor found in food. Apparently Greenspon's findings are of little value as will be shown later when the extrinsic factor and vitamin B_{12} are compared.

Johnson et al. (1942) presented evidence of a new chick-growth factor present in casein and liver meal. This factor was found to be distinct from vitamin A, thiamine, riboflavin, nicotinic acid, pantothenic acid, p-aminobenzoic acid, choline, and pyridoxine. The factor was soluble in ether and ethanol and was thermostable. Alfalfa leaf meal, ground yellow corn, wheat, and soybean meal were deficient in the growth factor(s).

Hammond (1942) reported that cow manure promoted growth of chicks when the diet was deficient in riboflavin. He found that cow manure contained a factor which stimulated comb growth in both males and females. In a later publication, Hammond (1944) found that dried cow manure and dried rumen contents were superior to alfalfa leaf meal in the simple, all-plant-protein, wartime diet. The diet contained sufficient quantities of vitamin A and riboflavin. Growth was not related to the riboflavin content of the cow manure or rumen contents, because there was a nutrient common to dried cow manure, dried rumen contents, and sardine fish meal which was not supplied by the all-plant protein diet. The androgenic effect of cow manure, which is objectionable for chicks intended to be used later for egg production or breeding, as reported earlier was eliminated simply by slow drying at a low temperature or drying at 80° C. or above.

Ross et al. (1942) studied reproductive processes of gilts and rats fed a growing-fattening ration of swine. This basal ration consisted of 76.35% ground yellow corn, 17.5% expeller soybean meal, 5% ground alfalfa hay, 0.5% iodized salt, and 0.65% calcium flour ($CaCO_3$). One group of gilts receiving the basal ration and another receiving the basal ration plus 10% additional ground alfalfa hay were bred and allowed to farrow. The gilts receiving the basal ration (5% alfalfa) failed to provide sufficient milk adequate for normal growth of their pigs. There was no inhibition of the reproductive function up to farrowing. The pigs during the lactation period became thin and emaciated with several dying before weaning. The ration containing 15% alfalfa meal permitted normal reproduction and lactation in the sows. The rats receiving the basal ration showed an impairment of reproduction during fetal development or suckling period. Intrauterine hemorrhages occurred with resorption or death of the fetuses from toxemia. Lactation was inadequate for those born alive. The ration containing 15% alfalfa meal produced normal reproduction and lactation in the rats even through the second generation in some cases.

Hill et al. (1944) found that certain combinations of commonly used feedstuffs for chick rations were not complete with riboflavin supplementation. These rations

required a growth factor(s) for maximum growth response. Heuser and Norris (1944) conducted an experiment with Leghorn pullets in which they divided the pullets into two groups with three pens per group and 25 birds per pen. The pullets were fed all-mash rations with one-half, three-fourths, and all of the supplementary protein (11.25%) from expeller soybean meal. The balance of the protein was meat scraps. One group (three pens) received 3% dried whey to increase the riboflavin. There was no difference in egg production among the six pens. Hatchability did not vary among the three pens in either series; however, the dried whey series showed approximately 20% higher hatchability than the other series. This was explained by the addition of riboflavin in the dried whey. In a second experiment, the addition of B-Y feed, source of riboflavin, to the series not receiving dried whey increased the hatchability up to that of the dried whey series.

McGinnis et al. (1944) used a solvent-extracted soybean meal as the only protein concentrate to Leghorn hens for seven weeks and failed to get hatchability above 50%. When soybean meal was replaced with 3% meat scraps, hatchability was almost as high as a commercial breeder mash. A marked increase in hatchability occurred when 6% meat scrap or 0.2% liver paste was used instead of soybean meal. Dried brewer's yeast or choline did not contain the unidentified

factor found in meat scraps or liver paste.

Cooperman et al. (1945) found that a factor(s) in whole liver was necessary at a level of 3% of the ration for optimum growth and blood regeneration in monkeys. After riboflavin was administered to deficient monkeys, a plateau below normal was reached for hemoglobin values, body weights, and for erythrocyte and leucocyte counts. Iron, 1:20 liver powder, extracted liver residue, or increasing the casein level to 24% was ineffective in restoring the blood picture and body weight to normal. Apparently some dietary essential in whole liver was removed from the extracted liver.

Whitson et al. (1945) checked various grains for growth-promoting factors in chicks. The chicks were grown to 10 weeks of age on diets consisting of 51.8% single grain, 35% soybean meal, 8% alfalfa leaf meal, 2% butyl fermentation solubles, a source of vitamin D, and appropriate mineral supplements. Wheat proved to be superior, in most cases, to corn, barley, oats, and milo as judged by rapidity of growth. A variation in the individual grains was found, because some lots of wheat were no better than some lots of corn and barley. The wheat, which supported most rapid growth, was lower in the protein content than other grain samples and this particular lot of wheat had been stored six years before using. The protein content and age of wheat were considered not to be the contributing factors. Three

per cent sardine meal or 8% dried cow manure supplied the factor(s) responsible for the superiority of wheat over corn according to the investigators. Their conclusions do not appear correct because more recent work has shown that wheat does not contain the factors necessary for chick growth found in sardine meal and cow manure. The quality of the protein in wheat may have accounted for the difference.

Mishler et al. (1946) found that nicotinic acid impaired the growth of chicks when 5 μg. or more were present in 100 gm. of a corn-soybean meal ration. A rapid growth was obtained in chicks fed the ration containing 55% corn and 40% soybean meal with supplements of riboflavin, choline, nicotinic acid (below 5 μg./100 gm.), pantothenic acid, simple mineral mixture and vitamin A and D fish oil. Supplements of animal protein were not essential for rapid growth when the nicotinic acid was kept below 5 μg. per 100 gm. of feed.

A growth-promoting factor for chicks has been found in hen feces. Rubin et al. (1946) obtained the same growth response in chicks by feeding a 5% urine-free hen feces ration as they did when a 5% cow manure ration was fed. A urine-containing hen feces ration stimulated growth slightly. The urine-free hen feces were secured by making artificial ani in the hens. The workers concluded that the growth-promoting factor for chicks was synthesized either in the

lower digestive tract or in the voided feces. Feces from three to six-week-old chicks did not contain measurable amounts of the growth factor.

Rubin and Bird (1946a) have presented evidence that the growth factor of cow manure (Hammond, 1942, 1944) is not identical with the growth factors of various lactobacilli from liver, yeast, fermentation residues, factors U, R, or S, vitamins B_{10} or B_{11}, synthetic folic acid, or pyracin lactone. Five and 8% levels of cow manure in the rations improved the growth-promoting properties of the basal diet, whereas the above-mentioned factors and ingredients did not. These same investigators (Rubin and Bird, 1946b) prepared concentrates of the chick-growth factor in cow manure. They found that the growth factor was soluble in water, 50% and 95% ethyl alcohol, but it was insoluble in chloroform and ether. The factor was stable to heat in a dry state at 100° C. for one hour. Autoclaving in solution for 15 minutes did not destroy the factor. It was found that the chick-growth factor can be transmitted from the hen through the egg to the chick. The rations containing 5% and 8% cow manure or fish meal transmitted enough of the factor to their chicks to permit adequate growth for at least six weeks when fed the basal diet containing protein entirely of plant origin.

Whitson et al. (1946a) conducted an experiment of 40 weeks' duration with hens fed a diet of corn and soybean meal with small quantities of alfalfa leaf meal, vitamins, and mineral supplements. When the level of soybean meal was increased from 0% to 40%, in increments of 10%, the hatchability decreased. There was a significant difference statistically between the hatchability of eggs produced by hens receiving no soybean meal and those receiving 20%, 30%, or 40%. The high levels of soybean meal did not affect egg production, body size, or egg size. Apparently, hens can tolerate soybean meal, at least to 40% of the ration. In another experiment of 48 weeks' duration by Whitson et al. (1946b) egg production was significantly impaired when 8% of dried cow manure of high androgenic potency was added to an all-mash ration. No change in egg production occurred when the cow manure had been dried at 80° C. for 24 hours. A ration high in soybean meal containing no animal protein was supplemented with 8% heat-treated cow manure. The cow manure improved hatchability.

During a 43-week period, Bird et al. (1946) fed hens a diet composed of 30% soybean meal as the source of protein. They found that 66% of the fertile eggs hatched as compared with 84% from hens receiving a good breeder mash containing sardine meal as the protein supplement. The diet containing 30% soybean meal was corrected by adding

5% cow manure, 10% sardine meal, or 10% dried skimmilk. The hatchability with these rations was 82%, 78%, and 79%, respectively. The soybean meal ration not only produced eggs of low hatchability but also chicks of low viability. Mortality in this group was 16% to 38% as compared with 1% to 11% for the controls.

Heuser et al. (1946) reported that hatchability was the poorest on soybean meal rations during winter and early spring but improved during the summer. Supplements of the known B-vitamins gave some benefit. The workers suggested the possible synthesis of the unknown factor by the hen or by microorganisms as Rubin et al. (1946) had concluded. A marked growth response in chicks was obtained when 2% to 3% fish meal was added to the soybean meal ration. Condensed fish solubles, liver paste, liver meal, choline, and folic acid also produced a growth response in chicks.

Bethke et al. (1946a) fed hens a ration containing soybean meal as the only source of supplemental protein. With this ration, egg production was satisfactory but hatchability was low. Factors necessary for hatchability were present in meat scraps, menhaden fish meal, dried skimmilk, and to a lesser extent in commercial casein and dehydrated alfalfa meal. Meat scraps and menhaden fish meal contained more of the hatchability factor(s) than dried skimmilk. At the same time Bethke et al. (1946b) reported

that a ration of yellow corn, oats, wheat by-products, dehydrated alfalfa meal, soybean meal, minerals, and adequate vitamins A, D, and riboflavin was deficient in the factor(s) necessary for good hatchability and 0.15% choline chloride and 0.15% or 0.20% DL-methionine, either alone or in combination, were not the limiting factor(s) in the ration. Five per cent meat scraps (not 2.5%), 2% dried pork liver, 1.5% liver extract, and 4% condensed fish solubles increased the hatchability of the eggs significantly when added to the ration. Dried pork liver, liver extract, and condensed fish solubles contained more of the essential factor(s) than meat scraps.

In another experiment, Bethke et al. (1947b) fed hens for 60 days the same basal ration as explained in a previous report (Bethke, 1946b). They found that the essential factor for hatchability was present in 2% sardine fish meal and 2% condensed fish solubles. The factor was not the following vitamins with numbers indicating mg. in each pound of ration: 7.6 pantothenic acid, 8.0 niacin, 1.6 pyridoxine, 0.7 folic acid, or 0.1 biotin.

Bird et al. (1947) held an inborn characteristic responsible for the variation of hatchability among hens receiving the same ration. They fed 183 hens a diet containing no animal protein for 11 months. Forty-four per cent of the hens produced eggs whose hatchability was

between 0% and 70%, 37% between 70% and 85%, and 19% between 85% and 100%. The high hatchability of the latter group could not be explained on the basis of coprophagy, storage of essential factor, or adaptation to an unfavorable diet. The chicks from these hens also showed high, intermediate, and low viability and growth rate to six weeks of age. The same group of workers (Groschke et al., 1947) demonstrated that seasonal variation was a contributing factor to hatchability and that cow manure corrected it. A corn-soybean meal basal diet was fed to hens of the same breeding. Hatchability was highly variable. The hens were then divided into two groups, high and low hatchability. The superiority of the former was maintained for a period of two years. The group with low hatchability in open-front houses showed seasonal variation each year with the hatchability improving during the summer and declining in the fall. A control group kept in batteries showed no increase during the summer. The addition of cow manure to the corn-soybean meal diet raised the hatchability of the low-hatchability group and eliminated the seasonal variation.

Bethke et al. (1947a) found that 4% sardine fish meal or 2% condensed fish solubles increased hatchability, and these materials contained a growth factor(s) which was transmitted from the hen through the egg to the chick. The latter finding is in agreement with the work of Rubin and Bird

(1946b) who fed cow manure at 5% and 8% levels and fish meal. Additional work by Rubin and Bird (1947a) supporting the finding that the growth factor is transmitted from the hen through the egg to the chick showed that more of the factor was deposited in the yolk than in the albumen. Another experiment demonstrated the presence of the growth factor in the acetone-insoluble fraction of the egg yolk. Hens fed 10% sardine meal produced eggs containing enough of the growth factor so that two yolks, but not one yolk, per kilogram of diet supported chick growth approaching the optimal rate.

In determining the effect of large quantities of soybean meal on chicks, Rubin and Bird (1947b) fed a ration containing 70% soybean meal to young chickens. Growth was inhibited and mortality was increased. Both effects were corrected by adding the growth factor of cow manure (acid precipitate of a water extract of dried cow manure) to the diet but 0.4% DL-methionine produced no change. The inhibition of chick growth from large quantities of soybean meal was not due to the heat-labile trypsin inhibitor found in raw soybeans. The cow manure improved the nutritional value of heat-treated soybean meal more than raw soybean meal.

McGinnis et al. (1947) showed that incubation of hen feces for 72 hours at 30° C. stimulated the synthesis of an

unidentified factor(s) required for maximum growth and livability of chicks. Feces that were frozen or collected immediately contained little or none of the factor. It was concluded that synthesis of the chick-growth factor takes place after voiding of the feces and not to any extent in the digestive tract. In this experiment the hens had been fed a ration of wheat, corn, soybean meal, and minerals. This ration was deficient in the factor(s) necessary for hatchability and chick growth. During the collection period of 36 hours, the outdoor temperature was at no time above zero Centigrade. The hens were kept in an unheated house.

The data presented by McGinnis and Carver (1947) reveal that few feedstuffs are able to furnish enough of the unidentified growth factor for the hen to supply their chicks any length of time. Chicks from hens receiving a basal diet containing soybean meal but no animal protein grew poorly and mortality was high. The addition of fish meal to the chick basal diet promoted growth and prevented mortality. The chicks from hens receiving a soybean-meal basal diet plus 1.7% fish meal grew better when fed the chick-basal diet than chicks from hens on their basal diet. Thus, some of the chick growth factor had been carried through the egg to the chick. Fish meal at 4.6% level plus the hen basal diet stored enough of the unidentified factor in the eggs to meet the chick requirements for maximum growth

and livability for four weeks. Fish meal at 5% level, 3% fish solubles, or 0.5% alcohol-soluble liver fraction in a ground pea diet stored the factor(s) to meet the chicks' need for four weeks. Little or no storage in the egg occurred when 15% dehydrated alfalfa meal was added to the pea diet. Some storage of the unidentified factor(s) took place when 2% B-Y riboflavin concentrate was added to the breeder ration. It appears that a good breeder ration should contain enough of the factor(s) to permit storage in the egg without the addition of riboflavin.

In the course of investigating the chick assay method for folic acid, Hill and Van Poucke (1947) concluded that the chick requires, in addition to the known required nutrients, at least one unidentified nutrient present in milk products, fish solubles and in widely variable amounts in gelatin. Nichol et al. (1947) found that concentrated liver preparations for the treatment of pernicious anemia patients were highly active in promoting growth of chicks. These preparations were as active as the crude liver preparations, and they showed greater activity when injected than given orally. One U.S.P. unit per day or 0.05 cc. gave a maximum growth response. There were no differences in the hemoglobin levels among the groups. It was impossible at that time (July 14, 1947) to decide whether or not the chick-growth factor was identical with the antipernicious

anemia factor in liver. The chick factor was soluble in 70% alcohol and precipitated with 95% alcohol. The investigators suggested that the chick may be a valuable assay animal for antipernicious anemia preparations.

Considerable progress had been made prior to 1948 regarding the growth and hatchability factor of chicks present in animal protein, but its identity had not been established. It was in 1948 when the isolation, crystallization, and further identification of the animal protein factor(s) were made.

Combs et al. (1948) presented evidence that the growth response in chicks and increased feed efficiency by the addition of 2% fish meal to a basal ration cannot be attributed to an increase in the essential amino acids. The results from this experiment show that fish meal contains a factor which is different than any of the recognized B-complex vitamins or amino acids required by the chick.

Kennard and Chamberlin (1948) demonstrated that built-up litter would serve as a source of the chick growth factor found in animal protein. Rubin et al. (1946) had previously shown that a 5% urine-free hen feces ration promoted chick growth comparable to that of a 5% cow manure ration. Kennard and Chamberlin obtained the same growth rate in 10-week-old pullets, when they were placed on built-up floor litter and receiving an incomplete ration

during a 15-week period, as those receiving a complete ration containing 10% meat scraps and not on built-up litter. The growth rate of day-old pullet chicks on built-up litter and receiving an incomplete ration was less than the chicks receiving the complete ration and not on built-up litter. The mortality was low in both groups. It was concluded, however, that built-up floor litter served as a source of the unidentified animal protein factor(s) necessary for the chicks receiving the incomplete ration. In a later publication, Kennard et al. (1948) found that built-up litter supplied the necessary factor to an all-plant protein diet for the production of eggs of maximum hatchability. Built-up litter lessened the requirements of animal and milk by-products and of certain vitamin products previously shown to be essential in the ration of hens on fresh litter (removed every two weeks) to produce eggs of high hatchability. The average hatchability of eggs from hens receiving the all-plant basal diet on fresh litter and on built-up litter (expressed in per cent) was as follows:

Diet	Fresh litter	Built-up litter
Basal	32	78
Basal + 2.5% dried whey	56	79
Basal + 2.5% dried whey + 2% meat scraps	67	80
Basal + 2.5% dried whey + 4% meat scraps	80	80

Thus, built-up litter was an excellent source of the hatchability factor.

Mishler et al. (1948) conducted a factorial experiment in which they found that 1.5% fish solubles in a soybean-meal basal ration was the best level for growth. The basal ration contained added riboflavin, choline, nicotinic acid, pantothenic acid, and methionine. Statistical analysis of the data revealed interaction between sex and fish solubles, the males giving greater responses.

Nichol et al. (1948) compared condensed fish solubles with other sources of the unidentified factor for chick growth. A ration containing 3% condensed fish solubles was comparable with a ration containing 3% whole liver powder in promoting an increased growth rate of chicks. Dried cow manure at a 3% level and 3% liver fraction L were good sources of the chick growth factor. Samples of dried "rumen contents extract" at 0.5 and 1.5% levels varied widely in their degree of activity. The same group of workers (Robblee et al., 1948a) reported that the degree of growth response in chicks was influenced by the hens' diet. A significant growth response in chicks was obtained by adding 3% condensed sardine fish solubles to a ration adequate in the known growth essentials.

Wiese et al. (1948) studied the variability of protein supplements in supplying the chick growth factor. Chicks hatched from hens fed a basal diet containing pea meal or pea meal and 2.0 and 4.0% meat meal grew poorly on a

soybean-meal basal diet adequate in the known chick growth factors. However, the addition of the meat meal or herring fish meal to the pea meal basal diet of hens reduced the chick mortality. When the hens' diet contained either 7.7% rendering plant meat meal or 9.2% packing plant meat meal, chicks were hatched with a marginal reserve of growth factor(s). Maximum growth was not obtained when these chicks were fed a soybean meal diet. A ration for hens containing 5.3% fish meal permitted enough of the chick growth factor to be transmitted through the egg to the chick to last four weeks on a ration deficient in the growth factor.

All-vegetable protein chick starter rations deficient in various growth factors have been investigated by Hill (1948). Soybean meal was found to be a variable source of one of the two unidentified nutrients needed in a cereal-soybean meal ration for optimum chick growth. Dried whey was a good source of the factor existing in variable amounts in soybean meal. Fish meal and 1% fish solubles (solid basis) contained the factor present in dried whey as well as an additional unidentified nutrient. Meat scraps were a source of the factor lacking in dried whey. Dried whey at 2,4,6, and 10% levels was superior to the all-plant basal ration but inferior to the fish meal ration at 4 and 6% levels. The feed efficiency (feed consumed per

unit of gain) for males at six weeks of age was 3.38 for all-plant basal, 2.87 for 2% dried whey, 2.89 for 4% dried whey, 2.69 for 6% dried whey, 2.70 for 10% dried whey, 2.69 for 2% menhaden fish meal, 2.65 for 4% menhaden fish meal, and 2.55 for 6% menhaden fish meal. The multiple nature of the deficiency in chicks on an all-vegetable protein diet, as shown in this review, suggests that animal proteins may contain several growth promoting factors and the "whey factor" as discovered by Hill is only one.

A remarkable discovery (reported April 16, 1948) was made by Rickes et al. (1948a) when they isolated a crystalline compound from liver (vitamin B_{12}) which produced positive hematologic responses in patients with pernicious anemia. This compound was administered to the patients in microgram quantities. At about the same time, Smith (1948a) in England (reported April 19, 1948) isolated two red pigments from ox liver. Both pigments were highly active in the treatment of pernicious anemia. In order to obtain approximately one gram of the red pigments, six lots of liver weighing four tons were processed. The pigments were soluble in water, not readily salted out, soluble in nearly anhydrous alcohol, in acetone containing a few per cent of water, and in glacial acetic acid. The pigments were insoluble in ether and chloroform. These discoveries have contributed a great deal toward the

advancement in studying the multiple nature of the animal protein factor.

Smith (1948b) was the first to report (July 1, 1948) that the antipernicious anemia factor isolated from liver contained cobalt. He made this discovery by examination of the ash. Smith postulated that if each molecule of the cobalt complex contained one atom of cobalt, the molecular weight would be 1500. In the event the complex lost 8% on drying the molecular weight would be 1600. His molecular weight assumptions have more recently been shown to be quite accurate.

A short time later (July 23, 1948) Rickes et al. (1948b) found that the red color of vitamin B_{12} was associated with its cobalt-complex character. By spectrographic examination, vitamin B_{12} was shown to contain phosphorus, nitrogen, but no sulfur. A microbiologic assay of an aqueous solution of vitamin B_{12} showed that autoclaving for 15 min. at 121° C. did not alter the activity within the range of experimental error. The cobalt ion (1 μg./ml.) did not stimulate the growth of Lactobacillus lactis as compared with the high potency of vitamin B_{12} (0.000013 μg./ml., half-maximal growth). Vitamin B_{12} (0.2 μg./ml.) in 0.015N NaOH solution was inactivated (microbiologic assay) at room temperature as follows: 20% in 0.67 hr., 45% in 6 hr., 90% in 23 hr., and 95% in

95 hr.; it was inactivated in 0.01 N HCl solution (10 μg./ml.) as follows: 18% in 3 hr., 75% in 23 hr., and 89% in 95 hr.

Ott et al. (1948) checked the activity of crystalline vitamin B_{12} for chick growth on day-old female White Leghorn chicks. Crystalline vitamin B_{12} (6 μg./kg.) added to a diet low in the animal protein factor stimulated the growth of chicks. Under the same conditions the optimal growth requirement appeared to be less than 30 μg. per kg. of diet. Since the crystalline compound and crude sources of the animal protein factor gave simular growth responses, the workers stated that it is possible for vitamin B_{12} to be identical with or closely related to the animal protein factor.

A nutritional factor associated with animal protein was found to be essential for the rat by Zucker et al. (1948). "Zoopherin" was suggested as the tentative name. The chick growth factor had already been named "vitamin B_{12}". Zoopherin is considered to be the same as vitamin B_{12}. The deficiency, after a natural lactation period, produced a marked growth restraint of the rats, high mortality, high blood urea, and a low white cell count. The material from liver or fish solubles used in rat curative tests was soluble in water, dilute acid and alkali, and dilute alcohol. It was moderately soluble in 95% alcohol and insoluble in petroleum ether and ethyl ether.

Seasonal variation in hatchability has been observed by Heuser et al. (1946) and Groschke et al. (1947). The latter corrected the seasonal variation by including cow manure in the ration. Groschke et al. (1948) believed that variation was due to coprophagy since summer was more favorable to synthesis of the essential dietary factor. The eggs from hens on a corn-soybean meal ration were highly variable with respect to hatchability. In an experiment lasting two years, these hens showed seasonal trends when kept in open-front houses with hatchability from 0 to 70%. Hatchability was higher in the summer than winter. Battery-kept hens showed no seasonal variation.

Petersen et al. (1948) also found that coprophagy played a part in improving the hatchability of eggs. Hens in open pens had better hatchability records than hens in batteries receiving the same rations. The results (expressed in per cent) are as follows:

Ration	Open pens	Batteries
Pea meal basal (hens, second year)	61.6	20.2
" " " (pullets)	51.4	24.5
" " " + 2.0% meat meal, (hens, second year)	80.5	53.6
" " " + 9.2% meat meal (pullets)	90.2	81.9
" " " + 5.3% herring fish meal (pullets)	89.1	85.5

In all cases the hatchability was higher in open pens indicating that coprophagy was the influencing factor.

A diet of yellow corn, oats, wheat by-products, dehydrated alfalfa meal, soybean meal, minerals, and adequate vitamins A, D, and riboflavin was found to be deficient in a factor essential for good hatchability by Pensack et al. (1949a). The essential hatchability factor was present in sardine fish meal, soluble in 80% hot ethanol, adsorbed by Darco G-60 at pH 3.0 from a water solution of an ethanol extract of fish meal, and not adsorbed at pH 3.0 from an 80% ethanol solution of a similar extract. The factor in sardine fish meal was transmitted from the hen through the egg to the chick. A ration containing 6% sardine fish meal caused a greater storage than 2% but neither was sufficient to meet the chick's maximum need to six weeks of age. The ration containing 2% sardine fish meal was as effective as the 6% in promoting hatchability. According to Pensack et al. (1949b) further work must be done to determine whether the growth factor(s) in fish meal is identical with vitamin B_{12} or is a combination of this vitamin and a closely related factor. These workers found that the chick growth factor in sardine fish meal and condensed fish solubles was soluble in 75% acetone and 80% ethanol. It was insoluble in n-butanol. The factor was colorless in solution.

Lindstrom et al. (1949) found that an all-plant basal diet containing 15% soybean meal did not support normal

hatchability when hens were kept on wire-floor laying batteries. Hens in open pens produced eggs of high hatchability by obtaining the hatchability factor by coprophagy. When the factor was supplied to deficient hens by feeding fish meal, hatchability increased rapidly within a period of 12 to 18 days. Hens that were transferred to a deficient diet after receiving the hatchability factor from fish meal for a period of six weeks retained sufficient amounts of the factor to produce eggs of high hatchability for 10 weeks.

In working with young pigs fed a corn-soybean meal basal diet, Burnside et al. (1949) showed that pigs responded little or not at all to a vitamin B_{12} charcoal concentrate, but pigs supplemented with an APF concentrate prepared from Aureomycin mash (fermentation product) responded markedly. The APF concentrate increased the feeding value of corn-peanut meal and corn-soybean meal rations to the point that these plant protein supplements were similar in feeding value to the fish meal used. When the APF supplement was added to the corn-peanut meal ration, the rate of gain was increased 2.13 times as compared with the control ration (2.26 times as calculated from the figures below). The rate of gain increased 30% when the corn-soybean meal ration was supplemented with the APF concentrate. The APF concentrate increased the rate of

gain a small amount when supplemented to the corn-fish meal ration. The average daily gain obtained from the basal rations and with the supplementation of APF concentrate is expressed in lb. as follows: peanut meal 0.62, peanut meal plus APF concentrate 1.40, soybean meal 1.01, soybean meal plus APF concentrate 1.31, fish meal (60.2% protein) 1.29, and fish meal plus APF concentrate 1.45. Additional work dealing with the levels of APF concentrate and of vitamin B_{12} for chicks and pigs will be covered later.

Various investigators have worked with vitamin B_{12} and called it by other names. Besides Zucker et al. (1948) calling vitamin B_{12} "zoopherin", Cary et al. (1946) called an unidentified rat-growth factor "factor X". Growth and development of weanling rats were affected if their diet was deficient in factor X but contained all known nutrients. It was found that young rats may be depleted in factor X simply by feeding their mothers a diet deficient in the factor. Casein was a good source of factor X. The growth of rats was retarded by feeding them casein extracted with hot alcohol. Supplementation of factor X-deficient rations with liver extracts, was water soluble, dialyzable, and precipitated with ammonium sulfate. In addition to casein and liver, Hartman and Cary (1946) showed that milk, dried skimmilk, cheese, beef and pork muscle, egg yolk, and certain leafy foods and feeds contained factor X. No

evidence of factor X was found in yeast, coagulated egg white, wheat bran, corn meal, linseed meal, soybean meal, and wheat flour. Factor X was required for growth, development, reproduction, and lactation of rats. Growth of rats was poor on diets containing lactose without factor X. High levels of protein were harmful to rats fed diets deficient in factor X. Death was the result in some cases.

Hartman et al. (1949) demonstrated that vitamin B_{12} functions like factor X even in animals on high protein diets. The workers postulated that the fundamental function of vitamin B_{12} in the normal mammal is in relation to the utilization of protein. Thus, evidence is presented that vitamin B_{12} and factor X are either identical or have the same function in the animal body.

Various liver preparations, as stated above, have been shown to be successful therapeutic agents in pernicious anemia. Subbarow (1945) stated that the amount of material needed by a pernicious anemia patient has decreased from 400 gm. of whole liver (17 years ago) to less than 1 mg. per day (400,000:1). This is largely due to the production of liver concentrates. More recently the discovery of vitamin B_{12} has caused this ratio to become wider than ever.

West (1948) reported a favorable response in three pernicious anemia patients by intramuscular injections of

3, 6, and 150 μg. of vitamin B_{12}. With the 150 μg. dosage, the reticulocyte count rose from 0.5 to 27.0% in 5 days and red cell count from 1,500,000 to 3,400,000 in 23 days. The other two patients also showed good responses. If one considers 100 μg. of vitamin B_{12} as a possible dosage for pernicious anemia patients, the ratio with 400 gm. of whole liver would be 4,000,000 to 1. Thus, vitamin B_{12} at 100 μg. dosage is 10 times more potent than liver concentrates. West in a personal communication to Rickes et al. (1948b) obtained negative results with the cobalt ion in two cases of pernicious anemia. Levels of 500 and 150 μg. of cobalt ion were injected subcutaneously as acetate and chloride, respectively. The average dietary intake of cobalt for adults has been estimated at 100 μg. per day. Hence, from West's findings, vitamin B_{12} cannot be replaced by the cobalt ion.

Stokstad et al. (1948) isolated a nonmotile, rod-shaped organism from hen feces which was capable of producing the animal protein factor. The organism was grown aerobically on simplified media containing no appreciable quantities of the animal protein factor. Concentrates of the material produced by the growth of the organism were highly active as a source of the animal protein factor by the chick assay method. The chicks received a diet containing all the known B-complex vitamins with high levels

of soybean meal or alcohol-extracted casein during the assay. These concentrates were also effective parenterally in producing a hematopoietic and clinical response in pernicious anemia patients. The workers stated that they were not sure that this substance is identical with the anti-pernicious anemia factor or vitamin B_{12}.

Prior to the isolation of vitamin B_{12}, folic acid was used in treating some cases of pernicious anemia. A positive hematologic response has been obtained in many patients with this therapy (Nutrition Reviews, 1948). Since the discovery of vitamin B_{12}, it has become clear that the action of folic acid is different than that of vitamin B_{12} in pernicious anemia. Liver extracts may not contain significant amounts of the free or conjugated folic acid. Consequently, folic acid may be indicated with liver extracts (vitamin B_{12}) in treating pernicious anemia patients. A deficiency of folic acid in these patients may have accounted for the hematologic response obtained by this therapy.

Berk et al. (1948) administered vitamin B_{12} to a pernicious anemia patient in relapse and "combined system" disease which developed during irregular treatment with synthetic pteroylglutamic acid (folic acid). A rapid and marked improvement in the neurologic and hematologic pictures occurred. Liver extracts (both pork and beef)

produced a severe local and systemic sensitivity reaction. Vitamin B_{12} did not produce the reaction. Conclusions should not be drawn from one patient; however, the results indicate that vitamin B_{12} may be effective against the neurologic as well as the hematologic manifestations of pernicious anemia.

Reisner et al. (1949) treated three pernicious anemia patients with 5 to 150 mg. of thymidine (thymine desoxyriboside). A slight reticulocytosis was present, but the red cell count remained unchanged. More will be stated later about thymidine in regard to its interference with some of the microbiologic methods of assay.

Macrocytic anemia of pregnancy has been shown to be refractory to vitamin B_{12} by Day et al. (1949). Over a period of 15 days, 135 units of liver extract were given intramuscularly with no increase in erythrocytes or reticulocytes. When 27.5 μg. of vitamin B_{12} were given over a period of 8 days, no increase in erythrocytes or reticulocytes occurred. Parenteral administration of pteroylglutamic acid gave an excellent hematopoietic and clinical response in this case of macrocytic anemia of pregnancy. An initial dose of 30 mg. of folic acid was given. The patient then received 15 mg. daily, except one day's rest each week, until delivery. The reticulocyte peak of 18% was reached on the sixth day. The red cell count approached normal. The investigators postulated

that folic acid deficiency is responsible for the development of macrocytic anemia of pregnancy.

Morgan et al. (1949) obtained hematologic responses in three cases of pernicious anemia in relapse by daily intramuscular injection of 20 mg. of a crude concentrate derived from 125 gm. of beef skeletal muscle. The concentrate, which was injected daily, was equivalent to 1.0 μg. of vitamin B_{12} as determined by microbiologic assay. The quantity injected appeared to be optimal in two patients and suboptimal in one. Morgan et al. stated that the activity obtained from beef muscle and liver concentrates appeared to be similar, and the activity is probably due to vitamin B_{12}. They also stated that the extrinsic factor of Castle in beef muscle appears to be identical with vitamin B_{12}. Work was cited in this report indicating that crystalline vitamin B_{12} is not altered by incubation with human gastric juice for 22 hours at a pH of 2, 3, and 7 as determined by microbiologic activity and examination of absorption spectra. Thus, evidence for a reaction between vitamin B_{12} and the intrinsic factor of Castle was not found in vitro.

Gardner et al. (1949) have recently shown that the simultaneous oral administration of vitamin B_{12} and the gastric (intrinsic) factor enhanced the erythropoietic effect as compared with vitamin B_{12} given alone orally.

The erythropoietic effect, however, was greater when the same amount of vitamin B_{12} was given alone parenterally. These investigators prepared a daily dose for pernicious anemia patients by extracting 400 gm. of beef skeletal muscle. This was made from an autoclaved, 70% alcohol filtrate of an aqueous extract of the beef muscle. The alcohol was removed by evaporation. The preparation was inactive orally in three patients with pernicious anemia. When the preparation was given orally with 150 cc. of normal human gastric juice, it was found to be moderately active. The preparation was still more active when given intravenously without gastric juice. Less than 1 μg. of vitamin B_{12} was present in each daily dose as determined by microbiologic assays and erthropoietic effects. This work suggests the possibility that the food (extrinsic) factor is identical with or chemically related to vitamin B_{12}. The workers, which included Castle, stated that the gastric (intrinsic) factor is essential merely for aiding the absorption of low concentrates of vitamin B_{12} present in certain foods other than, for example, liver.

The isolation of vitamin B_{12} has increased the number of investigators working with this vitamin. Recently, vitamin B_{12} has been credited with performing many functions, besides those mentioned above, in the animal body. Some of these functions have been worked out, whereas others

need further investigation.

A three-week procedure was standardized and used by Nelson and Evans (1948) in which they checked the efficacy of many dietary factors in improving the lactation performance of rats. A number of the B-vitamin concentrates contained varying amounts of the missing factor(s) for lactation. A 0.5% liver eluate powder was the best source tested in producing normal lactation. The workers stated that the liver eluate furnished a high level of folic acid. Perhaps the vitamin B_{12} content of the liver eluate powder was a contributing factor in improving the lactation performance of rats.

The feeding of desiccated thyroid to increase the requirement of the growing animal for some unknown nutrients had been suggested to Robbles et al. (1948b). This requirement was to be supplied by the feeding of the unidentified antithyrotoxic factor. An experiment using day-old chicks was run four weeks. A thyrotoxic condition (induced hyperthyroidism) was produced in the chicks by feeding desiccated thyroid or iodinated casein. A diet containing 3% condensed fish solubles or the injection of 1 U.S.P. unit of Reticulogen (Lilly), a concentrated liver extract used in the treatment of pernicious anemia, counteracted the thyrotoxic condition. Growth was stimulated by

adding either desiccated thyroid or iodinated casein to a ration adequate in the unidentified chick growth factor(s). An improved assay range for the unidentified chick growth factor(s) in condensed fish solubles and Reticulogen was produced by the addition of 0.125% desiccated thyroid or 0.02 to 0.03% iodinated casein to the basal ration. Thus, the antithyrotoxic factor may or may not be vitamin B_{12} since liver and fish solubles contain multiple nutrients.

In working with rats, Ershoff (1949) found that the antithyrotoxic factor was not identical with vitamin B_{12}. Growth was reduced in hyperthyroid rats by feeding casein, in a purified ration, as the dietary protein and sucrose as the carbohydrate. A water-insoluble fraction of liver completely counteracted the retardation in growth. Crystalline vitamin B_{12} was ineffective. Apparently, factors in casein and vitamin B_{12} are distinct from the factor in the insoluble fraction of liver.

Nichol et al. (1949a) obtained different results with chicks than Ershoff (1949) did with rats. Nichol et al. administered vitamin B_{12} orally and parenterally to chicks receiving a basal ration containing 0.05% iodinated casein. One-half maximal growth response was produced in the thyrotoxic chicks when the ration contained 0.75 μg. of vitamin B_{12} per 100 grams. When the vitamin B_{12} was doubled (1.5 μg./100 gm. of ration), the growth response compared

closely to the chicks receiving 3% condensed fish solubles. This growth response was considered to be close to the maximum. After a two-week test period, the chicks receiving 1.5 μg. of vitamin B_{12} per 100 gm. of ration showed a growth response (weight difference from control group) of 88 gm. as compared with 85 gm. for the group receiving 3% condensed fish solubles. An intramuscular injection of 0.1 μg. of vitamin B_{12} daily gave a growth response of 72 gm. as compared with 79 gm. for chicks receiving an injection of 0.5 μg. of vitamin B_{12} daily. The workers concluded that vitamin B_{12} can replace the APF activity of condensed fish solubles and injectable liver preparations in thyrotoxic chicks.

In working with hemoglobin regeneration, Nichol et al. (1949b) produced a severe anemia in chicks by intramuscular injections of 2.0 mg. of phenylhydrazine hydrochloride per 100 gm. of body weight. The chicks had been on a purified ration deficient in folic acid for a period of 14 to 21 days. Within two days the chicks had developed a severe anemia with a hemoglobin range between 1 and 5 gm. per 100 ml. of blood. In the experiment, chicks whose hemoglobin was between 2 and 4 gm. per 100 ml. of blood were distributed into comparable groups to study the effect of folic acid, liver extract, and vitamin B_{12} on hemoglobin regeneration. Liver extract or vitamin B_{12} alone did not affect the rate

of hemoglobin formation. Liver extract and folic acid caused a more rapid regeneration of hemoglobin than folic acid alone. It was found that vitamin B_{12} completely replaced liver extract in stimulating the formation of hemoglobin in the presence of folic acid. This work indicates that vitamin B_{12} does not have optimum activity unless folic acid is present.

Schaefer et al. (1949a) have shown that an interrelationship exists between vitamin B_{12} and choline or methionine when the latter functions as a choline precursor. A basal diet containing 0.3% methionine and 0.007% choline produced fatal hemorrhage in the kidneys of all the weanling rats within two weeks or less. The incidence of renal hemorrhage was markedly reduced by adding vitamin B_{12} concentrate or crystalline vitamin B_{12}. A diet containing 30 μg. of vitamin B_{12} per kilogram replaced about one-half of the supplementary choline or methionine required for protection against renal hemorrhage. When vitamin B_{12} was added to a diet containing a sub-protective level of choline, a significant increase in weight gain occurred. There was, however, no increase in weight gain when vitamin B_{12} was added to a diet containing adequate choline. From these data it appears that dietary choline has a sparing action on vitamin B_{12}.

In another experiment, Schaefer et al. (1949b) found

that the choline requirement of chicks was reduced by adding vitamin B_{12} to the diet. The chicks received a basal diet supplemented with 0.6, 0.2, 0.1, and 0.05% choline chloride. The weight gain at the end of two weeks of the vitamin B_{12} group over the control group was 26, 32, 63, and 21 gm., respectively. The diet containing 15 μg. of vitamin B_{12} per kg. with a choline level of 0.1% was as effective as the diet containing the vitamin B_{12} concentrate in a two-week feeding period. These investigators (1949c) also reported that chicks fed a ration containing 0.2% choline supplemented with 30 μg. of vitamin B_{12} per kg. did as well as chicks fed a vitamin B_{12}-free diet with 0.6% choline.

Becker et al. (1949) working with cobalt-deficient lambs obtained no significant response in weight gain or hemoglobin by intramuscular injections twice a week of crystalline vitamin B_{12} in quantities varying from 2 to 125 μg. per week. These injections were continued for a period of six weeks. When 1 mg. of cobalt was fed daily to cobalt-deficient lambs, there was an improvement in appetite along with hemoglobin concentration. There was also a gain in body weight. The intramuscular injection of the same quantity of cobalt into cobalt-deficient lambs evoked no response over a period of seven weeks. It was assumed that the injected cobalt did not reach the rumen in sufficient quantity for synthesis of vitamin B_{12}.

Mushett and Ott (1949) demonstrated that vitamin B_{12} prevents gizzard erosions in chicks and also lessens the severity of those affected. Chicks receiving a diet of 70% soybean meal and all the recognized nutrients with levels of vitamin B_{12} from 30 to 500 μg. per kg. of diet exhibited fewer and less severe gizzard erosions than chicks given the same diet without vitamin B_{12}. No difference in the severity of the lesions was observed in the gizzards of chicks receiving 30, 40, 80, or 500 μg. of vitamin B_{12} per kg. of diet. The gizzard erosions were almost completely prevented in chicks from hens fed a commercial breeder ration containing several animal protein products by adding 2.5 μg. of vitamin B_{12} per kg. of chick basal diet. Chicks from hens fed an all-vegetable ration required 10 μg. of vitamin B_{12} per kg. of basal diet to prevent gizzard erosions. No response in hemoglobin formation was found by including 30 μg. of vitamin B_{12} per kg. of basal diet. Fourteen chicks fed the basal diet had an average hemoglobin reading of 8.5 gm. per 100 ml. of blood, whereas 14 chicks receiving the basal diet plus 30 μg. of vitamin B_{12} per kg. of ration had an average hemoglobin reading of 8.4. The method for hemoglobin determination was not described. The incidence of gizzard erosions in chicks receiving the basal diet was 92% while those receiving the basal diet plus vitamin B_{12} supplement was 52%.

Ninety-five per cent of 20 unfed, day-old chicks had gizzards showing erosions. In order to obtain a maximum growth response in chicks from hens fed a commercial breeder ration, 2.5 μg. of vitamin B_{12} had to be included in each kg. of basal diet. Chicks from hens fed an all-vegetable protein diet required 30 μg. of vitamin B_{12} per kg. of basal diet for maximum growth.

Vitamin B_{12} has been reported by Popper et al. (1949) to inhibit hepatic injury produced by carbon tetrachloride in rats. The administration of 15 μg. per 100 gm. of body weight preceding acute carbon tetrachloride intoxication prevented fatty metamorphosis and depletion of cytoplasmic ribonucleic acid in the liver of rats. Ribonucleic acid has been shown to disappear early in hepatic injury.

Dempsey and Wislocki (1946) stated that the basophilic hue of the cytoplasm of liver cells is due to the presence of ribonucleic acid compounds. The basophilic inclusions increased in size and abundance after eating. These changes were demonstrable after staining with pyronin or methylene blue.

Stern et al. (1949) recognized the fact that basophilia in liver cells is determined by the ribonucleoproteins, so they studied the effect of vitamin B_{12} on liver basophilia. A basal diet containing 22% protein was fed to a group of weanling rats, a second group received the basal

diet plus 440 μg. of vitamin B_{12} per kg. of diet, and a third group received the basal diet with 4.2% of the soybean meal replaced with that quantity of whole dried liver. The rats receiving the basal diet grew poorly and showed little or no liver basophilia. The rats receiving the basal diet plus vitamin B_{12} or liver grew well and showed considerable cytoplasmic basophilia in the hepatic cells. The livers were fixed in Carnoy's fluid, dehydrated through alcohol, and sectioned 8μ in thickness. The sections were stained with eosin and methylene blue.

2. Chemistry

Prior to the discovery of vitamin B_{12}, various investigators determined some of the chemical characteristics of the rat growth factor, chick growth factor, and the hatchability factor. The chemical characteristics of these factors as observed by the workers were quite similar in most cases, e.g., being soluble in various percentages of ethanol, insoluble in butanol, ether, and chloroform.

Jaffe and Elvehjem (1947) fractionated the growth-stimulating factor in liver. They showed that the active principle in liver can be adsorbed on and eluated from norit, and it was soluble in ethanol and insoluble in butanol. Storage of liver extracts in the cold caused inactivation. Activity was retained in liver extracts after

heating in a boiling water bath for 10 minutes. Extracts of fresh liver were prepared which were active in stimulating the growth of rats. The extract was prepared by grinding 1 kg. of fresh liver, mixed with 2 liters of 95% alcohol, filtered through cheese cloth, residue pressed in filter press, residue treated twice with 1 liter of 60% alcohol, filtered, and pressed. The two filtrates were combined, reduced to 100 ml., extracted with ether three times, and filtered. One ml. was equivalent to 10 gm. of liver. In checking the activity of this filtrate, each rat received 3 drops daily which was equivalent to 1.5 gm. of fresh liver or 0.45 gm. of dried fresh liver. The liver-treated rats averaged 4 gm. more per week on purified diets than the controls and 7 to 10 gm. more per week on a corn-soybean meal diet than the controls.

Rickes et al. (1948c) compared the properties of a red crystalline compound isolated from a grisein-producing strain of Streptomyces griseus with those of vitamin B_{12}. The melting point, temperature that the crystals lost their red color, refractive indices, presence of cobalt and phosphorus, and solubility tests of the red crystalline compound from Streptomyces griseus were practically the same as those of vitamin B_{12}. The APF activity of the new compound for chick growth was 30 μg. per kg. of diet.

This is comparable to that found for crystalline vitamin B_{12} by Ott et al. (1948). Randolph West compared the clinical response of the new compound with vitamin B_{12}. He found that both compounds were very similar. These data indicate that the crystals from the microbiologic source and vitamin B_{12} are identical.

Ellis et al. (1949a) isolated a red crystalline compound from anahaemin, a proprietary name for the active hematopoietic principle of liver of Dakin and West, which is probably identical to that of Rickes et al. (1948a) and Smith (1948a). In an aqueous solution this compound showed a characteristic high absorption spectra. A main band appeared in the visible spectrum with a maximum at 500 m μ and a "shoulder" at approximately 520 m μ. Also, two distinct maxima occurred at 361 m μ and 278 m μ. The same authors (1949b) demonstrated the presence of phosphate in acid hydrolysates of vitamin B_{12}. The atomic ratio of cobalt to phosphorus in vitamin B_{12} was found to be 1:1.

Brink et al. (1949) found that vitamin B_{12} contains one atom of cobalt which is 4.5% of the vitamin B_{12} molecule. This corresponds to an approximate minimum molecular weight of 1300. An ebullioscopic molecular weight determination gave a value of 1490 plus or minus 150. An analysis of vitamin B_{12} indicates that its empiric formula may be but not necessarily limited to $C_{61-64}\ H_{86-92}\ N_{14}\ O_{13}\ PCo$.

The investigators showed that vitamin B_{12} is optically active (levorotatory) and shows absorption maxima at 2780, 3610, and 5500 Å. Vitamin B_{12} is also a polyacidic base. In an acid hydrolysate of vitamin B_{12}, no amino acids could be detected. It was concluded that vitamin B_{12} is not a polypeptide and does not appear to contain even one α - amino acid moiety.

Brink and Folkers (1949) reported that in an acid hydrolysate they have identified a new basic compound by its reactions and synthesis as 5,6-dimethylbenzimidazole. It was noted that 1,2-diamino-4,5-dimethylbenzene moiety was present in 5,6-dimethylbenzimidazole, vitamin B_{12}, and in riboflavin. Riboflavin, however, failed to yield any 5,6-dimethylbenzimidazole on hydrolysis.

A new crystalline product called "vitamin B_{12a}" has been produced by Kaczka et al. (1949) by catalytic reaction of vitamin B_{12} with hydrogen ions. This new compound has high hematopoietic activity in pernicious anemia patients, although it was less active than vitamin B_{12}. Vitamin B_{12a} had about one-half the APF activity of vitamin B_{12} in rats and 30% plus or minus 15% in chicks. The spectrum of this artificial product in an aqueous solution was similar to but different than that of vitamin B_{12}.

A crystalline fraction was separated from liver extract by chromatography by Pierce et al. (1949).

This compound had an absorption spectrum maxima different than those reported for vitamin B_{12} (Brink et al. 1949). Vitamin B_{12b} was suggested as a name for the new liver fraction since a compound closely related to vitamin B_{12} had been called vitamin B_{12a}.

3. Assay methods

A satisfactory method for the assay of vitamin B_{12} has not been discovered. Macrobiologic methods as rat, mouse, monkey, and chick take a much longer time than a microbiologic method. A number of compounds stimulate the growth of microorganisms which interferes with the determination of the vitamin B_{12} content of the material being assayed. Another disadvantage of the microbiologic method is the required digestion of natural foodstuffs before the assay can actually be made.

Cooperman et al. (1946) demonstrated the presence of a heat-labile substance in raw liver and raw milk which stimulated the growth of Streptococcus faecalis R on a purified medium. Autoclaving the liver or milk for 15 minutes at 15 lb. pressure destroyed the growth factor. The same group of investigators (Ruegamer et al. 1947) prepared two semisynthetic media containing all the essential nutrients for the growth of Streptococcus faecalis R. When small inocula of cold water extracts of liver

preparations, milk products, and extracts of certain grains were added to the media and short incubation periods used, growth and acid production by this organism were stimulated considerably. Leafy materials were poor sources of the growth factor. A good correlation was found between the microbiologic method and the monkey assay when crude preparations were assayed. Partially purified fractions showed no correlation.

In working with Lactobacillus lactis Dorner, Shorb (1947a) discovered that this microorganism failed to grow in an amino acid basal medium containing all the synthetic B vitamins. The addition of clarified tomato juice or liver extracts to the medium did not produce growth. However, if tomato juice and liver extracts were added to the medium at the same time, maximum turbidity and acid formation resulted. Shorb developed an assay method for the tomato juice factor (TJ) and liver factor (LLD). The LLD factor was considered not to be identical with any of the unidentified growth factors for bacteria.

In other publications, Shorb (1947b, 1948) reported that by assay the LLD factor is concentrated in refined liver extracts in the same relationship as the extracts alleviate the symptoms in pernicious anemia. This relationship suggested that the LLD factor might be the anti-pernicious anemia factor. The TJ factor was found to be

more concentrated in crude liver preparations than refined liver extracts. It was assumed that the TJ and LLD factors might be important in the treatment of pernicious anemia because both factors are required together for the growth of Lactobacillus lactis Dorner.

In working with Lactobacillus casei, Daniel et al. (1948) discovered that this microorganism required animal protein factor(s) present in liver extracts in addition to strepogenin for maximum growth. Strepogenin is a growth factor found in casein and tomato juice. Tomato juice, recently canned, contained enough of the APF and strepogenin for the growth of Lactobacillus casei. The APF activity of canned tomato juice decreased with age while the strepogenin activity remained unchanged. The APF activity was removed from tomato juice by adsorption on norit, whereas the strepogenin was found in the filtrate.

Shive et al. (1948a) isolated from hog liver a crystalline factor functionally related to folic acid. This compound at the time of isolation was thought to be thymine desoxyriboside (thymidine). Later, the compound was identified as thymidine. The factor was far more active than folic acid in promoting a half-maximum growth response of Leuconostoc mesenteroides. Extracts from liver, hog duodenal mucosa, and grass were highly active in promoting the growth of the microorganism. Milk, muscle tissue, and yeast were

poor sources. Liver extracts contained as much as 1% of the factor by the above method of assay.

Shive et al. (1948b) reported that thymidine can replace liver extracts containing antipernicious anemia principles (vitamin B_{12}) in the assay with Lactobacillus lactis Dorner. A half-maximal growth response was obtained with thymidine at a concentration of 1 to 3 μg. per 10 cc. of medium. Thymine did not produce a growth response at concentrations as high as 100 μg. per 10 cc. The workers postulated that vitamin B_{12} may function in the biosynthesis of thymidine since the latter replaced vitamin B_{12} in the nutrition of Lactobacillus lactis Dorner.

Snell et al. (1948) obtained similar results as Shive et al. (1948b) with lactic acid bacteria. Snell et al. found that thymidine was an essential growth factor for several of the lactic acid bacteria in media of known composition. Thymidine permitted the growth of several strains of Lactobacillus acidophilus. Again, thymine was inactive in stimulating growth of microorganisms, and the media contained an excess of folic acid.

Wright et al. (1948) made similar observations with lactic acid bacteria. When 0.4 to 2.0 μg. of thymidine were added to each tube, growth was comparable to that observed with liver. Thymine was without activity under these

conditions. From these data, the investigators concluded that vitamin B_{12} functions as a coenzyme in the conversion of thymine to thymidine because Lactobacillus lactis does not require vitamin B_{12} in the presence of thymidine. Wright et al. also stated that the primary biological defect in pernicious anemia may be the inability to synthesize certain nucleosides (thymidine) from parent purines and pyrimidines.

Shive et al. (1948c) found that purines or their derivatives and thymidine were essential for the growth of Lactobacillus lactis Dorner in the absence of vitamin B_{12}. Thymidine could not be replaced with thymine; however, a number of the purines were interchangeable. Guanylic acid was the most effective purine. Mixtures of adenine and guanine or of hypoxanthine and guanine were practically as active as guanylic acid. The workers discovered that APF can be replaced with thymidine for the growth of Lactobacillus leichmannii. This organism required folic acid in a medium containing purines for growth. Thymine replaced the folic acid after a lag in growth. APF was replaced by thymidine in the presence of folic acid, and after a lag phase thymidine replaced the folic acid. Shive et al. were of the opinion that folic acid and APF have independent functions, which vitamin B_{12} handles alone,

in the biosynthesis of thymine and thymidine. Skeggs et al. (1948) reported that Lactobacillus leichmannii responded well to purified liver preparations and also to large amounts of thymidine. They suggested that in the assay of crude materials, thymidine must be considered.

The most recent microbiologic assay for vitamin B_{12} is one in which thymidine does not interfere. Hutner et al. (1949) described an assay method using the algal flagellate, a free-living protozoan, Euglena gracilis var. bacillaris. Half-maximum growth response was obtained with 0.01 mμg. of crystalline APA (antipernicious anemia) factor (vitamin B_{12}) per ml. Thymidine was without activity up to 10 μg. per ml. This free-living protozoan (Nutrition Reviews, 1949) grows in the presence of light in a single medium containing inorganic salts, potassium citrate, sodium butyrate, monosodium glutamate, and two vitamins, viz., thiamine and the crystalline antipernicious anemia factor (vitamin B_{12}). It was stated (July 1949) that the specificity of this method for natural products containing vitamin B_{12} needs further testing.

Two assays for vitamin B_{12} using mice have been reported by Bosshardt et al. (1949). One method used growing mice from mothers receiving a diet free of vitamin B_{12}. The other method involves the ability of vitamin B_{12} to counteract the retarded growth of mice that were fed

thyroid-active materials. By these methods, liver contained APF (vitamin B_{12}) for growth, but yeast and wheat germ did not. APF improved the retarded growth of mice receiving thyroid-active materials. It was found that APF was transmitted from the mother to her young during gestation or lactation or both, and it may be stored by the animal for some time. When the maternal diet was deficient in APF, a high mortality of young mice occurred one to three days post partum.

Register et al. (1949) used the rat in assaying material for vitamin B_{12}. The rats were placed on a basal diet free of vitamin B_{12} for a depletion period of two weeks. During the next two weeks the material being assayed was fed. The growth response during the last two weeks was used as a criterion in evaluating the vitamin B_{12} content of the material. Beef skeletal muscle was found to contain twice as much vitamin B_{12} as normal pork skeletal muscle while abnormal pork muscle contained only a trace or no vitamin B_{12}. The higher content of vitamin B_{12} in beef muscle was explained by rumen synthesis. The pig is a monogastric animal.

Recently (Nov. 1949), five other substitute growth factors for vitamin B_{12} have been recognized for the growth of Lactobacillus leichmannii by Winsten and Eigen (1949). A bioautographic method in studying these growth

factors revealed marked similarities among parenteral liver preparations, condensed fish solubles, and a fermentation product containing APF. The APF activity of condensed fish solubles has been thought to be due to vitamin B_{12} since growth was promoted in chicks and in lactic acid bacteria. The workers demonstrated a factor in condensed fish solubles that does not "move" on a paper chromatogram as vitamin B_{12}. Two other more rapidly moving growth factors than vitamin B_{12} were observed in other sources of vitamin B_{12}. The investigators suggested that all sources of vitamin B_{12} should be checked by bioautographic studies to determine the types of growth factors present.

Jukes (1949) reported that the major deficiency in a chick diet and assay can be corrected by supplying vitamin B_{12}; a second growth factor present in an APF concentrate is needed for maximum growth. Seven groups of chicks with 12 in each group gave the following results:

Supplement per kilo of basal diet	wt. in gm.	No. of chicks surviving at 25 days
None	108	6
15 μg. vitamin B_{12}	254	12
25 μg. vitamin B_{12}	268	12
50 μg. vitamin B_{12}	270	11
2 gm. APF conc.	275	12
3 gm. APF conc.	294	10
5 gm. APF conc.	316	12

Jukes stated that Cary and Hartman first noted this

phenomenon. Similar observations were made later by both Bird and McGinnis. Thymidine and other desoxyribosides do not interfere with the chick-assay method as they do in the lactobacilli assays. Jukes is of the opinion that the chick assay method is the most reliable at the present time (Nov. 1949) for measuring the potency of APF supplements.

4. Recommended dietary levels for chickens and swine

The vitamin B_{12} content of feedstuffs is known to vary with each lot. For instance, the vitamin B_{12} content in tankage and meat scraps may vary depending upon what organs and tissues make up the products, method of processing these products, and also the nutritional status of the animals from which these products came. Therefore, the recommended levels for natural products in a crude form are hard to determine. More accurate recommendations as to what levels of purified extracts or concentrates to feed can be made by first using an assay method. At the present time one must consider the assay method and also the possibility of interfering compounds in making the determinations. As will be shown, there is considerable variation in the recommended levels of crystalline vitamin B_{12}. Therefore, it appears that more work should be done in establishing accurate levels for optimum growth in the

animals requiring dietary vitamin B_{12}.

With natural feeds, Lillie et al. (1949) observed no apparent change in growth, egg production, and hatchability in pullets receiving 4% fish meal in a basal ration containing 40% soybean meal until eight weeks of age with the group being divided and placed on range for 22 to 24 weeks. One group received 2.5% fish meal on range and the other group received only the basal ration containing no animal protein. Thus, the critical period of the vitamin B_{12} requirement for pullets is during the first eight weeks of life. If ample vitamin B_{12} is supplied during this time, the vitamin can be eliminated later from the diet provided the pullets are placed on range. The results with turkeys on wire floor show that vitamin B_{12} cannot be reduced at four weeks of age but can be reduced (not eliminated) at eight weeks. Poor growth was obtained when poults received 6% fish meal for four weeks and then 6% meat meal in comparison with poults receiving 6% fish meal for eight weeks and then 6% meat meal. Poults receiving 6% fish meal throughout the experiment were comparable with the latter group. Meat meal had to be fed at an 8% level to be an effective supplement.

Stokstad et al. (1949) found that a level of 15 μg. of vitamin B_{12} per kg. of diet or 0.3 μg. injected intramuscularly each week supplied the vitamin B_{12} requirement

of chicks. However, maximum growth was not obtained until other supplements were added such as 20 or 60 gm. of Streptomyces aureofaciens mash per kg. of diet. The oral potency of vitamin B_{12} was 50% that of the injected potency. As stated previously, Ott et al. (1948) discovered that optimum growth in chicks was obtained when a diet low in the animal protein factor was supplemented with 30 μg. of crystalline vitamin B_{12} per kg. of diet.

Johnson (1949) produced chicks equal in growth and appearance with those receiving a ration containing animal protein by adding an APF concentrate to an all-plant ration. Chicks fed a simple ration containing 23% plant protein with liberal amounts of vitamins A, D, riboflavin, pantothenic acid, niacin, and choline without the addition of animal protein or APF concentrate had an average weight of 1.5 lb. at eight weeks of age. Chicks receiving the same diet plus APF concentrate (0.5%) weighed 1.8 lb. at eight weeks of age while chicks receiving the Iowa State College broiler ration weighed the same amount. One pound of crystalline vitamin B_{12} was considered enough for 900 million chicks for one week.

During an eight-week period, Neumann et al. (1948) added Reticulogen (an antipernicious anemia liver extract) to a synthetic milk diet fed to pigs. Growth rate was increased by feeding Reticulogen. The pigs receiving this

extract averaged 19.96 and 21.6 kg. as compared with 13.53 and 16.0 kg. for the controls. The hemoglobin, red, and white cell counts were normal in all pigs at the end of the eight-week period.

Catron and Culbertson (1949) reported that the vitamin B_{12} requirement of growing-fattening pigs is around 10 μg. per lb. of ration. Vitamin B_{12} at levels of 5, 10, and 15 μg. per lb. of this ration did not affect the amount of feed required to produce 100 lb. of pork. In comparison with the all-plant ration, 10 μg. of vitamin B_{12} added to each lb. of ration saved from 7 to 10% of the feed in producing 100 lb. of pork. The ration containing the vitamin B_{12} increased the daily gains from 19 to 24%.

Luecke et al. (1949a) determined the effect of a vitamin B_{12} concentrate on the growth of weanling pigs fed a corn-soybean meal ration. The pigs were fed a basal ration consisting of 77% corn, 20% soybean meal, and a 3% complex mineral mixture. The following B-vitamins were added as expressed in mg. per lb. of feed: thiamine 5, riboflavin 5, calcium pantothenate 20, niacin 25, and pyridoxine 2. Choline was added at 0.1% level, and 2,000 I.U. of vitamin A and 200 I.U. of vitamin D were added to each lb. of ration. One group of pigs received the basal ration alone while a second group received the basal ration plus 0.5% vitamin B_{12} concentrate (10 μg./lb. of ration).

This concentrate contained 2 mg. of vitamin B_{12} per lb. as measured by the chick assay method. The pigs receiving the basal ration gained 0.87 lb. per day while the vitamin B_{12} group gained 1.20 lb. per day. The basal group consumed 2.57 lb. of feed per day in contrast to 3.21 for the vitamin B_{12} group. The vitamin B_{12} group utilized the feed better (2.65 lb. of feed for each lb. of gain) than the basal group which required 2.94 lb. of feed for each lb. of gain.

Cunha et al. (1949) studied the effect of vitamin B_{12}, APF, and soil for the growth of pigs. A charcoal-vitamin B_{12} concentrate containing 2 mg. of vitamin B_{12} activity per lb., an APF fermentation product, and soil (top 3 inches of soil, strained of vegetable matter, and dried at room temperature) were added to a basal ration consisting of 57% yellow corn, 41.5% peanut meal, and the necessary minerals and vitamins to meet the pig's requirements. The soil added at the 5% level was beneficial to the pigs which indicated that some unknown factor(s) was present. A gain of 26% occurred in pigs averaging 33 lb. by adding 1.1% APF concentrate to the ration. When smaller pigs were used, the rate of gain was increased 2.5 times that of the basal group with 2.2% APF. The addition of 0.2% vitamin B_{12} concentrate to the basal ration was of no benefit. During the third week the vitamin B_{12} concentrate was raised to 0.4%. The rate of gain decreased. From these results,

APF must contain more than vitamin B_{12}. The workers stated that Catron and Culbertson (1949) found vitamin B_{12} to be beneficial when added to a corn-soybean meal, alfalfa, mineral ration to which vitamins A and D were added. They explained that their results may be different than Catron and Culbertson on the basis of using peanut meal instead of soybean meal and leaving out the alfalfa. If this is true, the ration used by Cunha et al. appears to be deficient in some of the essential growth factors.

Hogan and Anderson (1949) took six pigs from their mothers at two days of age and placed them in cages with wide-mesh wire floors. They were raised on synthetic milk which consisted of vitamin-free casein, sucrose, corn starch, lard, minerals, and generous supplies of vitamins A, D, E, K, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, choline, folic acid, biotin, and inositol. Intramuscular injections of crystalline vitamin B_{12} were given to three pigs at three-day intervals until they were 38 days old. A total of 50 μg. of vitamin B_{12} was given to one pig, 100 to a second, and 200 to the third pig. The six pigs grew at about the same rate during the first six weeks. In the following four-week period, the pigs having received the vitamin B_{12} injections made an average gain of 26.8 lb. while the others gained 15.9 lb. During the following six-week period, one pig without vitamin B_{12}

died unexpectedly and one did not gain consistently. The third pig started to decline so 15 μg. of vitamin B_{12} were injected. It began to gain at a moderate rate. The three pigs receiving vitamin B_{12} grew at a uniform rate with an average gain of 58.8 lb., which was considered exceptional for that age.

Neumann et al. (1949) found that 42 μg. of vitamin B_{12} per kg. of synthetic milk containing soybean protein improved growth and the physiological well-being of baby pigs. A maximum growth response was produced and hematopoiesis was improved. Symptoms of vitamin B_{12} deficiency in pigs were extreme irritability, sensitiveness to touch, sluggish movements, and inability to stand on hind legs.

A ten-week experiment with weanling pigs was conducted by Anderson and Hogan (1949) to determine the value of a vitamin B_{12} concentrate in rations containing practical feedstuffs. The largest and most thrifty pigs were fed the corn-soybean basal ration; the smallest and least thrifty pigs were fed the basal ration plus 10 μg. of vitamin B_{12} per lb. of feed; and the pigs, intermediate in size and thrift, were fed a ration containing fish meal. The latter ration was believed to be nutritionally complete. The average daily gain for the basal group was 0.69 lb., vitamin B_{12} group 0.82 lb., and fish meal group 0.92 lb. During the last four weeks of the experiment, the average

daily gain for the basal group was 0.86 lb., vitamin B_{12} group 1.14 lb., and fish meal group 1.36 lb. Thus, vitamin B_{12} certainly can be used to an advantage with practical feedstuffs.

Hale and Lyman (1949) fed a vitamin B_{12} concentrate to young growing pigs. The basal ration consisted of 70% yellow corn, 25% soybean meal, 3% alfalfa leaf meal, 1.5% pulverized limestone, and 0.5% salt. One group of pigs received the basal ration, and another group received the basal ration plus 1.5 mg. of vitamin B_{12} per 100 lb. of feed (15 μg./lb. of ration). The pigs in each group were fed in individual pens. The pigs receiving vitamin B_{12} made 31% greater gains than the control group and also required 22.7 lb. less feed per 100 lb. of gain than the control group.

Lepley et al. (1949) reported that the addition of 0.2 to 0.5% APF concentrates to a basal ration consisting of yellow corn, soybean meal, dehydrated alfalfa meal, minerals (trace too), and vitamins A, D_2 and B-complex produced 19 to 24% faster gains in pigs. These gains equalled or surpassed the gains obtained by adding 2% condensed fish solubles or 6% meat and bone scraps. From these data, the tentative vitamin B_{12} requirement for growing-fattening swine was stated to lie between 5 and 10 μg. per lb. of ration. Two per cent condensed fish solubles had previously

been shown by these workers to be equivalent to 4% fish meal, 5 or 6% meat scraps, or 6% dried corn distillers solubles in an all-plant ration for growing-fattening swine in drylot (Catron and Culbertson, 1949)

Luecke et al. (1949b) studied the effect of a vitamin B_{12} concentrate on the growth of weanling pigs. A basal ration consisting of 77% corn, 20% soybean meal, 3% minerals, vitamins A, D, plus liberal amounts of thiamine, riboflavin, calcium pantothenate, nicotinic acid, and pyridoxine was fed to two lots of pigs with seven pigs in each lot. To one lot of pigs a charcoal-adsorbed-vitamin B_{12} concentrate was added at a 0.5% level. These pigs were farrowed from gilts which had never received any source of APF. In 11 weeks the pigs receiving vitamin B_{12} made an average gain of 1.5 lb. per day with feed efficiency of 2.66. The pigs receiving the basal ration averaged 0.98 lb. per day and required 2.90 lb. of feed per lb. of gain. A second experiment was conducted using the same rations as the first experiment with nine pigs in each lot which were farrowed from gilts having been fed animal protein and raised on pasture. In this experiment the vitamin B_{12} concentrate was adsorbed on fullers' earth instead of charcoal. At the end of six weeks the pigs receiving the basal ration made an average gain of 0.71 lb. per day while the pigs receiving vitamin B_{12} averaged 1.0 lb. per day. It is quite

obvious that vitamin B_{12} increased the daily gain and even improved the feed efficiency at the end of six and eleven weeks. These factors are very important in reducing the cost in producing a pound of pork and also in having hogs ready for market to take advantage of the seasonal rise in hog prices.

5. Additional growth factors

Factors other than vitamin B_{12} have been shown to stimulate the growth of tissues. The source of these factors may be closely related to vitamin B_{12} and yet their chemical characteristics are entirely different.

Briggs et al. (1943) discovered two dietary factors in liver and other materials essential for chicks. They are distinct from folic acid. One factor is essential for proper development of feathers which was named vitamin B_{10} and the second chick growth factor was called vitamin B_{11}. Both factors were found to be soluble in water, adsorbed on Norit and Superfiltrol at pH 3, eluted with a mixture of water, alcohol, and ammonia, and separated partially by fractional precipitation with ethyl alcohol.

Evidence has been presented by Sprince and Woolley (1945) that strepogenin, a bacterial growth factor, is a part of the protein molecule and not a contaminant. Thus, they believe that it is possible for a protein to contain

all the amino acids and still not have strepogenin activity due to the incorrect linkage or combination of amino acids. This, they feel, may explain the absence of strepogenin from egg white. Crystalline insulin, crystalline trypsinogen, crystalline trypsin, crystalline chymotrypsin, crystalline ribonuclease, crystalline tobacco mosaic virus, certain crystalline proteins of yeast, hemoglobin, and casein were found to be excellent or good sources of strepogenin. Dialyzed egg white, salmine, and gelatin were poor sources of strepogenin.

Woolley (1946) fed mice a highly purified ration using casein hydrolysate, tryptophan , and cystine as the source of nitrogen. The mice grew at a submaximal rate. Growth was promoted by adding small amounts of proteins rich in strepogenin, the peptide-like growth factor. Proteins low in strepogenin did not stimulate growth. Tryptic digests of casein were as active in promoting growth as the intact protein. Egg white being low in strepogenin did not promote growth until the ration was supplemented with an excellent source of strepogenin. Later, Woolley (1947) discovered that strepogenin required a free amino group in its structure to promote growth. By destroying the amino group in strepogenin-rich trypsinogen, casein, and insulin, their potency was either destroyed or altered.

Scott et al. (1947a) have shown that strepogenin and

factor S are probably identical as determined microbiologically with Lactobacillus casei. Chicks on a factor S-deficient diet failed to grow, became weak and anemic, and usually died before they were eight weeks old. Chick experiments assaying protein sources, as casein, egg albumen, and liver meal, for strepogenin and factor S showed that they are probably identical. In checking the nutrition requirements of the bacterium, Lactobacillus casei, Scott et al. (1947b) found that in addition to the known growth essentials, this microorganism requires strepogenin, a factor associated with animal products, and glutathione to maintain an optimum growth rate during the first 16 hours of incubation. These investigators believed that the vitamin requirements of this bacterium are very similar to those of animals.

Various derivatives of glutamic acid have been studied by Woolley (1948) for their ability to replace strepogenin in the growth of Lactobacillus casei. The peptides having strepogenin potency were serylglycylglutamic acid, glycylserylglutamic acid, alanylglycylglutamic acid, glycylalanylglutamic acid, and glycylglutamic acid. Several other glutamic acid derivatives with the substituted amino group were inactive. Serylglycylaspartic acid was antagonistic to the growth promoted by strepogenin. The growth-promoting peptides did not approach the growth promoted by natural

substances. Serylglycylglutamic acid, the most active, had a potency of one while the potency of casein and insulin concentrates was 40.

Novak and Hauge (1948a) reported a factor in distillers' dried solubles essential for the growth of rats. This factor was stable to heat, acid, and alkali. It was soluble in ether, ethanol, and water at widely different pH values. Phosphotungstic acid and lead acetate precipitated the factor. It was not adsorbed on fullers' earth or Darco. Florisil, Lloyd's reagent, Norit, and Decalso adsorbed the factor from acid solution. The workers stated that the factor is distinct from vitamin A, D, thiamine, riboflavin, pantothenic acid, niacin, inositol, p-aminobenzoic acid, choline, 2-methyl-naphthoquinone, pyridoxine, biotin, and folic acid. Later, Novak and Hauge (1948b) obtained this new rat growth factor in a non-crystalline but highly purified form from distillers' dried solubles, rice polishings concentrates, and liver extract. They called this factor vitamin B_{13}. Its absorption spectrum exhibited a maximum at 2820 $\mathring{A}$. This factor being soluble in chloroform and ether differentiates it from vitamin B_{12}.

A still more recent vitamin has been isolated in crystalline form by Norris and Majnarich (1949a) from human urine (33 mg. from 100 liters). This vitamin was named vitamin B_{14}. It increased cell proliferation in

vitro and hemopoiesis in vivo. By analysis the vitamin contained 19.6% nitrogen and 4% phosphorus but no sulfur or cobalt. Vitamin B_{14} was found to be about 10,000,000 times as effective in inhibiting cell proliferation in vitro in a suspension of tumor cells as xanthopterin. The latter compound is known to accelerate the proliferation of bone marrow cells and to inhibit neoplastic cells. A single injection of 0.01 μg. of vitamin B_{14} was as effective in correcting the anemia and leucopenia of anemic rats produced with sulfathiazole as a single injection of 1 mg. of xanthopterin. In another publication, Norris and Majnarich (1949b) reported that vitamin B_{14} may serve as a part of the intrinsic factor of Castle. Cell proliferation produced by vitamin B_{14} in bone marrow culture was equal to the rate produced by an incubated mixture of enzymes from rat gastric mucosa plus folic acid and xanthopterin. The products formed by the action of oxidase systems on xanthopterin and folic acid were believed not to be identical with vitamin B_{14}. The extrinsic factor of Castle was considered not to be a single factor and that xanthopterin, folic acid, teropterin, and possibly other pteroyl derivatives may serve as the extrinsic factor. Thus there are three vitamins or vitamin-like substances (vitamin B_{12}, "whey factor" of Hill, and vitamin B_{14}) associated with animal protein which have been

discovered as a part of the animal protein factor(s).

B. Chicken Blood

1. Bleeding methods

Various methods have been used in obtaining blood from chickens. Perhaps the wing-vein method is the most common. The site of the venous puncture may be made at any place along the course of the subcutaneous veins. Cutting a spike of the comb has also been used. Hamre and McHenry (1942a) obtained blood in their studies from a wing vein and also from a spike of the comb.

Andrews (1944) reported a method of obtaining blood directly from the heart. He prepared a canula from a 16-gauge California style bleeding needle by cutting off the pointed end at the ring. A short-beveled point was then ground on the blunt end of the needle to facilitate piercing the body wall between the fifth and sixth ribs at the junction of the vertebral and sternal portions. After the heart is felt by the canula, an 18-gauge needle, three inches long, is passed through the canula directly into the heart. This method was considered an improvement over the method described by Sloan and Wilgus (1930) in which a similar procedure was used but the canula omitted.

Some workers have obtained blood from the heart by passing a needle through the anterior entrance of the

pleural cavity,e.g., MacArthur (1950). Hofstad (1950) has recently reported a method of heart bleeding which does not require an assistant or extra equipment. The bird is placed on a waist-high table. The right side of the bird is placed down with the bird's back facing the operator. The right wing falls over the edge of the table adjacent to the operator and the left wing is then placed in apposition with the right wing. The wings are held against the table by pressure from the operator's body which holds the bird firmly and frees the operator's hands to carry out the bleeding process. In bleeding, the needle pierces the skin one inch behind the point of the keel and high enough to pass through the angle formed by the sternum and metasternum. The needle is directed at a 45 degree angle in an anterio-medial direction toward the opposite shoulder joint. By this method blood can be obtained quickly with low mortality.

2. Anticoagulants

There are a number of anticoagulants used in securing blood samples. Rourke and Plass (1929) used 1 mg. of heparin for 15 cc. of blood (human). They found heparin to be an ideal anticoagulant for blood sedimentation studies because it had no effect on the settling rate of the erythrocytes. They stated that electrolytes may bring

about instability of the blood.

In contrast with the work of Rourke and Plass (1929), Heller and Paul (1934) found that heparin had a tendency to shrink the erythrocytes of chickens; therefore, the cell volume is always slightly less than normal. Small blood clots had a tendency to form around heparin particles because heparin is slow to dissolve. It was also found that sodium, potassium, and lithium salts caused a decrease in cell volume, whereas ammonium salts caused an increase in cell volume. A mixture of 40% potassium oxalate and 60% ammonium oxalate (0,2% solution of the mixture) gave a minimum cell volume variation. DeVilliers (1938) working with ostrich blood discovered that lithium citrate produced slight crenation (shrinkage)of erythrocytes. Wintrobe (1946) found that the anticoagulant used by Heller and Paul was the most satisfactory.

3. Hemoglobin values; erythrocyte, leucocyte, and differential counts

A number of methods have been employed in determining the hemoglobin content of blood. The most accurate methods are those using a photoelectric colorimeter. The Dare and Tallqvist methods are based upon the direct matching of unchanged blood with color scales while the methods of Sahli, Sahli-Hellige, Newcomer, Wintrobe, Haden-Hausser,

and Osgood-Haskins convert hemoglobin into acid hematin. A visual comparison is then made between the standard and unknown. The methods involving color matching and visual comparison are less accurate, more inconsistent, and, in most cases, give slightly higher readings with avian blood than a photoelectric colorimeter. In making hemoglobin determinations with chicken blood, a problem is presented which is not encountered with mammalian blood. Hemoglobin readings, higher than they actually are, are obtained due to the nucleated erythrocytes. If a correction is not made or reading adjusted in some manner, one must realize that this reading is high. A comparison of hemoglobin readings among groups of birds being treated differently can be made without making the correction if one is interested only in the comparison and not in the actual hemoglobin concentration.

Dukes and Schwarte (1931) worked out a correction factor for the high hemoglobin values obtained with chicken blood. They used the improved Newcomer method. Their correction was to offset the turbidity formed in the acid hematin solution which they thought was caused by the abundance of nuclear material in chicken blood. The hemoglobin values (gm./100 cc. of blood) before correction were 16.4 for cocks, 12.3 for hens, 11.4 for pullets, whereas after correction the values were 13.5, 9.8, and 8.9, respectively.

Schultze and Elvehjem (1934) avoided the turbidity in the acid hematin solution by adding the blood to 0.4% NH_4OH and then acidifying the solution with concentrated HCl. This gave a clear acid hematin solution. The acid insoluble material collected in a small globule which did not interfere with the reading. Schultze and Elvehjem obtained the idea of using 0.4% NH_4OH from Palmer (1918). Palmer mixed 0.05 cc. of human blood with 5 cc. of 0.4% NH_4OH and compared this mixture with a standard carbon monoxide hemoglobin solution in a Duboscq colorimeter.

Holmes et al. (1933) used a modified Sahli method with the Osgood-Haskins new permanent standard and Sahli-Haskins tubes. A 100% reading on the tube was equivalent to 13.8 gm. of hemoglobin per 100 cc. of blood. The blood of six to eight birds out of a group of 30 was checked for hemoglobin at 3, 6, 9, and 12 weeks of age. The ration contained 19% protein of plant and animal origin. The hemoglobin content of the blood was about 9.0 to 10.0 gm. per 100 cc. of blood. The hemoglobin level of cockerels was found to be 0.3 and 0.4 gm. higher at 3 and 12 weeks of age, respectively, than pullets and that restriction of water intake during crate feeding did not increase the hemoglobin level of chicken blood. These data were not subjected to statistical analysis. It appears doubtful that the 0.3 and 0.4 gm. difference between males and females would be

significant when so few determinations were made.

Olson (1935) found that the photoelectric colorimeter and the Newcomer hemoglobinometer were in close agreement in measuring hemoglobin of chicken blood. The Dare and Tallqvist methods were not accurate. Olson (1937) obtained a mean of 9.11 gm. of hemoglobin per 100 cc. of blood from 453 observations on 20 adult female chickens and a mean of 11.76 from 280 observations on a group of 12 adult male chickens. No difference in sex was found in the hemoglobin values between 89 males and female chicks ranging in age from a few weeks to eight months. Olson analyzed the data statistically and concluded the following:

> (1) the hemoglobin and erythrocyte values of the blood of normal adult chickens vary with the season and are higher in the male than in the female, (2) the factor of environment has no effect on the values of the following constituents of chicken blood; hemoglobin, erythrocytes, thrombocytes, and the percentage value of lymphocytes and granulocytes, (3) the total leukocyte counts of chickens kept outdoors are higher than those of similar chickens kept indoors, (4) there is no significant difference between the total leukocyte counts of male and female chickens or of young and old chickens, (5) the lymphocyte values for female chickens are higher than those for male chickens, and the heterophil values are higher for the male than for the female, (6) the lymphocyte values for young chickens are higher than those for adult chickens, and the heterophil values are higher in the adult than in the young chicken, (7) seasonal factors do not influence the total or relative percentages of leukocytes or number of thrombocytes, (8) in general the percentage of monocytes is higher in chickens kept outdoors than in those kept indoors, and male chickens have a higher per cent of monocytes than females, (9) the

values for erythrocytes, leukocytes, and hemoglobin of young chickens are comparable to those for adult female chickens, (10) young chickens have slightly higher thrombocyte counts than adult chickens.

Harmon (1936) made 15,000 hemoglobin determinations on 3,000 chickens over a period of three years with a Dare hemoglobinometer. Realizing that this method is not very accurate, the large number of determinations certainly must have helped to establish hemoglobin levels accurate enough to conclude that the hemoglobin of chicken blood varies greatly from hatching time to maturity. The hemoglobin in chicks was high at hatching time and gradually decreased to reach a low at two weeks of age and then increased slowly to maturity. There was no significant difference in hemoglobin between sexes until eight weeks of age. Egg production decreased the hemoglobin, and broodiness or cessation of egg production caused an increase in hemoglobin. Chicks used for bleeding developed a longer bleeding time and a longer coagulation time with lower hemoglobin; however, their blood plasma proteins and uric acid were higher than normal chicks. The bleeding time was the period in which the blood continued to flow after the wing vein was punctured to obtain blood for hemoglobin determinations.

No difference in hemoglobin, erythrocytes, thrombocytes, leucocytes, differential, and packed erythrocytes was observed by Hamre and McHenry (1942b) in 18 hens fed

an adequate laying ration and 21 hens fed the same ration plus a small quantity of yeast-fermented mash. It was concluded that feeding of yeast-fermented mash made no difference in blood values. The hemoglobin was determined by the acid hematin method using a Klett-Summerson photoelectric colorimeter. The determination was made without attempting to avoid the turbidity of the acid hematin solution nor were corrections made for the turbidity.

Bankowski (1942) compared the Dare, Haden-Hauser, Newcomer as modified by Schultz and Elvehjem, Sheard-Sanford, and the acid and alkaline hematin photelometric methods. Duplicate samples for all methods were taken on 101 White Leghorn chicks six to eight weeks of age. The modified Newcomer method and the new photelometric acid hematin method gave the most consistent results and were considered the most reliable for chicken blood. The average hemoglobin for six-week-old chicks was 9.71 gm. per 100 ml. of blood by the two recommended methods and 9.83 for eight-week-old chicks. The Dare, Haden-Hauser, and Sheard-Sanford methods were considered unsatisfactory. The photelometric alkaline hematin method gave hemoglobin values averaging 2.25 gm. per 100 ml. higher than the two preferred methods.

Blood studies on 10 normal chicks, four weeks of age, were made by Campbell et al. (1944). The chicks receiving

the normal broiler ration had an average body weight of 184 gm. The hemoglobin averaged 8.7 gm. per 100 ml. of blood. The red and white cell counts averaged 2,350,000 and 28,900 per cu. mm. of blood, respectively. In another experiment, the same workers (1945) made blood studies on 20 normal four-week-old chicks. The hemoglobin was 7.74 gm. per 100 ml. of blood with a red cell count of 2,270,000 per cu. mm. of blood. The total white cell count was 29,935 per cu. mm. of blood. In this total white cell count there were 22,406 lymphocytes, 688 monocytes, 447 eosinophils, 659 basophils, and 5,733 heterophils. The hemoglobin was determined by the Evelyn colorimeter.

Gardner (1947) has summarized the values of the different blood cells and hemoglobin reported from 1936 to 1946. The average number of erythrocytes in the blood of adult chickens, regardless of sex or strain, was found to be 2,860,000 per cu. mm. of blood. The hemoglobin varied from 6.13 gm. per 100 ml. of the blood for four-week-old chicks (Evelyn colorimeter) to 15.27 by the Dare hemoglobinometer. The red cell count of chicks 10 days old was 2,300,000 per cu. mm. of blood and 3,600,000 for adults. Most workers reported a shift of the heterophil-lymphocyte ratio with age, a greater number of heterophils appearing in the blood of very young chicks and a preponderance of lymphocytes in the blood of adult chickens. The following is an analysis

of the white blood cells of adult chickens regardless of sex or strain:

	Per cent	Range
Heterophils	23.42	17.6-27.4
Eosinophils	1.93	1.9- 2.0
Basophils	1.63	1.5- 1.7
Lymphocytes	63.79	58.2-70.7
Monocytes	9.10	8.2-10.2

The average number of leucocytes in the blood of adult chickens, regardless of sex or strain, was 27,080 per cu. mm.

In studying five methods for the enumeration of erythrocytes and leucocytes in chicken blood, Olson (1935) found that Wiseman's method was more accurate for red cell counts and gave slightly higher counts than the other methods. The other methods were rated in this order, Blain, Shaw, Toisson, and Folkner. In counting leucocytes, Toisson's method gave more consistent results than the other two direct methods (Blain and Shaw). Wiseman's method was more suitable than Folkner's method (indirect methods). Olson rated the methods for counting leucocytes in this manner, Blain, Toisson, Wiseman, Shaw, and then Folkner.

Palmer and Biely (1935a) used Shaw's method and showed that the erythrocyte and leucocyte counts fluctuated around a certain level characteristic of the individual. Variations in total erythrocyte and leucocyte counts, other than those due to technique, were those peculiar to the individual, physiological condition of the bird (intake of

food or water, egg production, and environmental temperature) and possibly the time of day.

In another publication, Palmer and Biely (1935b) reported that in making blood counts, the time interval between the last feeding and the time of bleeding should be considered. They recommended that a standard procedure should be followed in feeding and making the counts. An increase in the erythrocyte counts was found in 31 birds out of 39 when the blood was drawn previous and subsequent to 48 hours of starvation. In eight birds there was a decrease. All birds showed a decrease in the leucocyte counts except one. Twisselmann (1939) showed that changes in differential counts of chicken blood could not be correlated with age in normal chickens. He used supravital and Wright's staining techniques.

4. Sedimentation rate

The sedimentation rate is not specific for any particular disease. It is, however, a reflection of the physiological condition of the animal. Rourke and Plass (1929) in finding heparin to be the ideal anticoagulant for blood sedimentation studies in humans stated that the aggregation of erythrocytes in sedimentation tests is a completely reversible phenomenon. Blood can stand at room temperature for 6 to 12 hours without the sedimentation rate being

affected. Longer standing slowed the rate; therefore, it was concluded that all tests should be completed the same day as the blood is drawn. (Part of the observations made by Rourke and Plass was later shown to be incorrect by other investigators.) Centrifuging blood for 20 min. at 2500 r.p.m. did not alter the sedimentation rate after remixing. Diluting blood with its own plasma increased the settling rate of the erythrocytes. Aeration of venous blood did not alter the sedimentation rate. An increase in temperature from 20 to 38° C. increased the sedimentation rate although the changes observed within the range of ordinary room temperature were hardly significant. The changes in the sedimentation rate due to the ingestion of food were slight. Short, violent exercise produced an insignificant and variable effect on the sedimentation rate. The workers stated that the accelerating effect from the increase in fibrinogen was compensated for by the retarding effect of the increase in cell volume.

One of the factors increasing the settling rate of erythrocytes is a decrease in number. Walton (1933), therefore, corrected the red cell count to 5,000,000 per cu. mm. by dilution or concentration. Then he constructed a graph whereby the corrected reading could be obtained after the sedimentation rate had been determined. This method was not considered accurate and is not recommended when the

erythrocyte count is less than 3,000,000 per cu. mm. of blood in humans.

Cherry (1934) studied the factors influencing the sedimentation rate of erythrocytes in humans and found that cell volume and the variations of fibrinogen (referred to as fibrin in the publication), euglobulin, and globulin in the plasma were the controlling factors. The fibrinogen nitrogen, euglobulin nitrogen, and globulin nitrogen were increased in both the slow and fast sedimentation groups, but the nitrogen content was greater in the fast group. A fall in the albumin nitrogen in the fast sedimentating group was at the expense of the rise in fibrinogen and euglobulin nitrogen. Leucocytosis also increased the sedimentation rate, but this was considered to be due to the increased fibrinogen content of the blood. Leucopenia caused no change in the sedimentation rate.

Boerner and Flippin (1935) found that correcting the erythrocyte concentration in humans before conducting the sedimentation test, as suggested by Walton (1933), enhanced the value of the test by making the blood comparable to normal individuals. The investigators concluded that it was best to run the sedimentation rate in humans within one hour after the sample is taken. A delay of two, three, or more hours decreased the sedimentation rate.

In working with bovine blood, Ferguson (1937)

demonstrated that the sedimentation rate was very slow as compared with the blood of humans and other mammals. The mean sedimentation rate in seven hours was 2.394 mm. Statistically, there was a highly significant difference between individuals as compared to that within individuals.

Kracke (1941) stated that the factors influencing the sedimentation rate are the number of red cells available for sinking, tendency toward segregation, and the physical and chemical composition of the plasma. Thus, a tendency toward autoagglutination or rouleaux formation of the erythrocytes increases the sedimentation rate. The sinking of the red cells or aggregates of these cells displaces the plasma which forms a current in an upward direction hindering the sinking of the clumps of cells. The sedimentation rate will, therefore, be faster in plasma with a greater viscosity. Fibrinogen and globulin are known to have a greater viscosity than serum albumin. Thus, the work reported by Cherry (1934) in which he found an increase in fibrinogen and globulin nitrogen associated with rapid settling of the erythrocytes agrees closely with the concept that greater viscosity hastens sedimentation. The sinking rate of red cells is increased by bubbling oxygen through citrated blood and decreased when carbon dioxide is passed through the blood. These statements do not agree with those of Rourke and Plass (1929). Kracke stated that

delay, as well as dilution, slows the settling rate of red cells. Therefore, cell volume determinations should be made shortly after obtaining the blood sample and the sedimentation rate should be corrected to compensate for the variation in the number and size of erythrocytes.

Coffin (1941) cited a number of diseases or conditions increasing the sedimentation rate of erythrocytes. Among these are acute general infections, malignancy, arthritis, local suppurative processes, pregnancy, and the anemias. Factors accelerating the sedimentation rate are a long sedimentation tube, tube with a large bore, inclination of tube from the vertical, reduced amount of anticoagulant, and a high room temperature. Factors decelerating the sedimentation rate are a short tube, a tube with a small bore, delay in starting the test, a large amount of anticoagulant, and a low room temperature. Coffin mentioned that the erythrocytes of horse blood settle rapidly, whereas the erythrocytes of cattle, sheep and goats are extremely slow and little or no settling occurs in the usual allotted time. With swine and dogs the sedimentation rate is intermediate.

5. Hematocrit

The hematocrit readings can easily be determined by centrifuging the sedimentation tubes the required length

of time following the sedimentation test. Heller and Paul (1934) found that the cell volume becomes constant for cow's blood in 65 min. at 1800 r.p.m. or 45 min. at 2200 r.p.m. and for chicken blood in 40 min. at 1800 r.p.m. To assure maximum packing of the erythrocytes, the sedimentation tube should be centrifuged again at the same speed for a short time to see that the packed cell volume remains constant. When the optimum speed and time are determined, consistency should be followed in all subsequent blood samples.

Terzian (1941) reported the volume of packed red cells of six normal chicks weighing 50 to 80 grams. The average was 27.45% with a range between 25.8 and 29.6%. The hematocrit tubes were centrifuged at 1200 r.p.m. for 30 min. In using the direct capillary hematocrit method for cell volume determination, Hamre and McHenry (1942a) discovered that heparinized blood gave accurate cell volume values after standing one hour although it was unsatisfactory for the enumeration of erythrocytes, leucocytes, and thrombocytes. As stated earlier, Hamre and McHenry (1942b) observed no change statistically in the packed red cell volume by feeding a small quantity of yeast-fermented mash to hens receiving an adequate laying ration.

Campbell et al. (1944) obtained an average hematocrit reading of 32.5% packed erythrocytes from 10 normal

four-week-old chicks. The same workers (1945) reported that the volume of packed red cells from 20 normal four-week-old chicks averaged 31.2% with a range between 27 and 36%.

C. Histopathology

1. Polarized light

According to Muñoz and Charipper (1943), the polarizing microscope was invented in France by Nachet between 1833 and 1855. At first its significance was not appreciated; however, its use has expanded until now it is an indispensable instrument in the fields of biological chemistry, mineralogy, organic and inorganic chemistry, ceramics, botany, and other sciences. As early as 1854 Virchow and other investigators used the polarizing microscope in the study of nerve tissue. They noticed that myelin sheaths of nerve fibers were optically active.

Polarized light is known to vibrate in one plane only, while ordinary light vibrates in all directions perpendicular to the direction in which it travels. Substances appearing black under the polarizing microscope, when crossed prisms are used, are called isotropic. When substances appear light under the same conditions, they are said to be anisotropic or show the property of birefringence.

A normal myelin sheath appears light when crossed prisms are used. Cellular detail, such as nodes of Ranvier, nuclei of Schwann cells and incisures of Schmidt-Lantermann, can be demonstrated in normal nerves with polarized light as they are isotropic. Schmitt (1939) stated that this sheath is really more complex than myelin. The lipid constituents of myelin are laid down in a neurokeratinogenic matrix. This albuminoid protein is isotropic; however, the abundance of lipids in the myelin sheath prevents the identification of the protein material with polarized light.

According to Schmitt and Bear (1939), the myelin sheath of vertebrates is produced by the Schwann cells which are ectodermal in origin. The Speidel theory of myelin formation states that myelin is formed by the co-operative metabolic activity of Schwann cells and the axone itself. Regardless of its formation, myelin consists of phospholipids, glycolipids or cerebrosides, sulfolipids, and sterols (Mathews, 1939). Normal myelin is practically void of true fats.

Myelin, a mixture of compound lipids, changes from its normal anisotropic appearance to an isotropic state when it becomes degenerated. The exact chemical change in this reaction appears to be unknown. Sutton et al. (1934) realized, however, that myelin may change to triglycerides, which are isotropic, thus permitting a more accurate study

of myelin sheath degeneration with the polarizing microscope.

Myelin degeneration, sometimes referred to as Wallerian degeneration, is present in many nutritional deficiencies, infectious diseases, and may be produced surgically by severing myelinated nerves. Baird (1936) stated that one and one-half hours after the severing of such nerves, degeneration can be seen by using the polarizing microscope. After three hours, the fibers are swollen and the myelin has broken into masses. With ordinary staining techniques, the condition would have to be prolonged three days before degeneration would be evident. Baird was apparently referring to the fat stains, such as Marchi or the Sudan stains, but they were not specified.

Sutton et al. (1934) produced myelin sheath degeneration of peripheral nerves in rats by feeding a vitamin A-deficient diet. The degeneration was arrested by the supplementation of vitamin A. External symptoms, such as paralysis and prostration, were not corrected with vitamin A therapy. In a later paper, Setterfield and Sutton (1935) stated that degeneration of the myelin sheaths of the sciatic and femoral nerves began 3 to 6 days before symptoms of avitaminosis A were present. They believe that one-fourth to one-third of the nerve fibers are affected before incoordination takes place.

Lee and Sure (1937) obtained results similar to those of Sutton et al. (1934). They produced myelin sheath degeneration in the sciatic nerves of rats by feeding thiamine-deficient, vitamin A-deficient, and B-complex-deficient diets. In these groups there were various degrees of myelin sheath degeneration. These results indicate that a deficiency of one or several substances may cause or contribute to the degeneration of the myelin sheaths in peripheral nerves.

Prickett et al. (1939) found a marked difference in myelin sheath degeneration of rats by varying the thiamine intake. In acute deficiencies the nerves approached normal, while rats on a suboptimal level of thiamine over a longer period of time showed considerably more myelin sheath degeneration. Control groups receiving an adequate amount of thiamine but limited in food intake showed more degeneration than rats receiving thiamine-deficient diets. The administration of thiamine to rats showing severe symptoms proved to be of little value. Early administration of thiamine to those rats showing mild symptoms was successful. Prickett et al. concluded that the tissues were damaged beyond repair when the rats did not respond to thiamine therapy.

Swenson (1950) concluded that the etiology of myelin sheath degeneration is not limited to thiamine deficiency.

It is true that animals showing degeneration of the myelin sheaths of peripheral nerves may be deficient in thiamine, but the primary cause of the degenerative change is some other factor. This statement is based on the observation that rats receiving an adequate amount of thiamine showed as much myelin sheath degeneration as those on thiamine-free and thiamine-deficient diets. This finding was observed in the sciatic nerves of rats stained by the Marchi method and also by viewing the sciatic nerves between crossed prisms in a polarizing microscope. Both methods checked closely with one another.

2. Tissue stains

The staining of tissues for histologic study aids in distinguishing the abnormal tissue from the normal. Various stains have been employed; however, the hematoxylin and eosin method has been the principal stain used in studying tissue changes microscopically. A universal stain to show maximum differentiation in all tissues of the animal body has not been developed. Thus, it becomes necessary to use different stains to obtain the desired effect in each type of tissue. For instance, hematoxylin and eosin stain may be used to study vascular and cellular changes in the nervous system while it does not demonstrate degenerative changes in the sheaths of myelinated nerves. Fat stains,

such as Marchi, Sudan, and Weigert, are used to detect these degenerative changes in myelin. Such stains are also used to determine fatty changes in other tissues, such as the liver and kidney. The transformation of normal myelin to triglycerides is shown by the fat stains and with polarized light in unstained myelinated nerves.

Hassin (1940) stated that 24 hours after a nerve is cut, the myelin becomes swollen, irregular, and breaks up into fragments. These fragments stain black with osmic acid which signifies degeneration. Baird (1936) found that degeneration could be detected with polarized light one and one-half hours after the nerve was severed. It is apparent that polarized light will detect degeneration sooner than the Marchi method in myelinated nerves.

Zimmerman (1940) reported that the peripheral nerve lesions in thiamine deficiency are non-inflammatory. He found that the primary change is medullary sheath destruction which is followed by axone disintegration. Setterfield and Sutton (1935) observed black droplets in the nerves of thiamine-deficient rats by using the Marchi stain. This finding checked closely with their polarized light study. From these observations, it appears that these workers may have been using rations deficient in factors other than thiamine.

Phillips and Engel (1938) demonstrated with polarized

light and by the Marchi method that a riboflavin deficiency in chicks may cause histologic changes characterized by degeneration of the myelin sheaths in the peripheral nerves and the spinal cord. Muscle fibers were also degenerated. Riboflavin therapy was successful when the degenerative changes were mild.

Various investigators have considered the Marchi method unreliable. Artifacts in the stained tissue can be produced by alterations in the technique. When the artifacts are present, the histopathology may be concealed. Duncan (1931) observed black droplets in the myelin sheaths of the controls as well as the diseased animals. This pathologic change was seen in the cat, rabbit, rat, and man. When the method was varied, the size, number, and general appearance of the black droplets were changed. Duncan believed that the Marchi method was valuable in denoting fatty changes in some tissues, but it was not specific for Wallerian degeneration. The diets for the animals showing degeneration were not given. There is a possibility that these diets were not balanced; therefore, the myelin degeneration may have been expected. The human tissues were obtained from aged individuals. Regardless of the conflicting results from the Marchi method, it still has its place in the staining of tissues.

D. Recommended Nutrient Allowances for Chicks

The Sub-Committee on Poultry Nutrition of the National Research Council (1944) has used the term "allowances" in preference to the term "requirements" because the latter term implies the quantity required for normal development and production and does not include margins of safety to offset the loss in manufacture, transportation, and handling of poultry feeds. For example, the margin of safety allowed by the National Research Council (1944) for vitamin A was 66%, vitamin D 45%, and 20% for the water-soluble vitamins. The National Research Council (1946) raised the allowance of vitamin A for starting chicks 67% of that prescribed in 1944. With the discovery of vitamin B_{12} and other nutrients, it appears that the recommended levels as suggested in 1946 should be revised to be in accord with the greater growth responses being obtained today.

The nutrient allowances for chicks as recommended by the National Research Council (1946) for each per pound of feed are as follows:

The amino acid requirements for chicks as given by Almquist (1947) and the National Research Council (1946) are expressed in per cent of ration as follows:

Amino Acid	Almquist	N.R.C.
Arginine	1.2	1.0
Lysine	0.9	0.9
Methionine, or	0.9	0.9
{Methionine	0.5	0.5
{Cystine	0.4	0.4
Tryptophan	0.25	0.25
Glycine		1.0
Threonine	0.6	
Phenylalanine	0.9	
Leucine	1.4	
Isoleucine	0.6	
Histidine	0.3	
Valine	0.8	

In studying the tryptophan requirement of chicks, Wilkening et al. (1947) found that 0.18% tryptophan was sufficient for normal chick growth. They stated that this lower level, in comparison with 0.25%, may be accounted for by the amount of nicotinic acid or other dietary components in the ration.

The fat content of ordinary rations usually meets the requirement for poultry. Morrison (1948) states that the minimum fat requirement for poultry is much lower than the amount found in ordinary rations, which contain 2 to 3% fat or more.

Russell et al. (1940) compared the growth rate of chicks to 14 weeks of age on a diet containing less than 0.1% fat with the control group receiving a ration

containing 4.1% fat. The fat of the ration containing less than 0.1% had been extracted with ether. This ration was supplemented with all the known dietary factors removed in the ether-extraction process. No significant difference was observed in the growth rate between the two groups. The chicks receiving the low fat diet utilized crystalline carotene although the diet contained five times the minimum requirement.

In studying the absorption and retention of carotene and vitamin A, Russell et al. (1942) concluded that the hen can absorb about 50 to 60% of carotene in the free form or from plant tissue on a normal ration containing 3.83% fat. Hens absorbed less crystalline carotene on a diet containing 0.07% fat than the hens receiving the normal ration. The absorption of vitamin A ester by hens was essentially the same on normal and low fat rations. The retention of vitamin A ester in the liver was greater in the hens receiving the normal fat ration than the low fat ration.

Morrison (1948) cited additional work in which the fat content of rations was increased to 20% of the ration by adding corn oil or soybean oil. As a rule, rations containing 10% or more of fat were detrimental to poultry. Egg production was lowered, growth retarded, and feed consumption was decreased. Rations containing 8 to 10%

fat gave inconsistent results with poultry. From these observations, it appears that the fat in ordinary rations composed of natural feedstuffs will take care of itself. The fat content of most natural rations will probably be between 2 and 7%.

Fiber is of major importance in poultry due to the relatively small digestive tract and also the limited ability to digest fiber. According to Morrison (1948) and Ewing (1947), a majority of workers have preferred a fiber content of not over 5 to 8% of the ration for chicks. Excessive fiber in the ration results in inferior gains and reduced feed efficiency. Also, some workers believe that vices in chicks such as feather picking or cannibalism may be caused by an insufficient amount of fiber in the ration.

III. MATERIALS AND METHODS

One hundred and five New Hampshire Red chicks were obtained from the Poultry Department of Iowa State College which were hatched from 200 eggs. These eggs were produced by hens which were maintained on an all-plant-protein ration for approximately the last five months. Fifteen chicks were helped out of the shells in order to obtain a sufficient number for the experiment.

The entire group of chicks received an all-plant-protein basal ration (Table 1) for two days. During this time the chicks were wing-banded and assigned at random according to a table of random numbers (Snedecor, 1946) to nine groups with 10 chicks in each group. The nine groups were then assigned at random to nine electric brooders manufactured by Oakes Mfg. Company, Tipton, Indiana. In addition to the basal ration, a second ration (Table 2) was fed which was identical with the basal ration except 3% of the soybean meal was replaced with 3% liver and glandular meal. The liver and glandular meal was manufactured by Armour and Company, Chicago, Illinois. The liver and glandular meal contained the following:

Crude protein, not less than	60%
Crude fat, not less than	6%
Crude fiber, not more than	3%
Nitrogen-free extract, not less than	0%

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Ingredients	Amount	Protein	Fat	Fiber	Ca	P	Choline	Vit. A	Vit. D
	%	%	%	%	%	%	mg.	I.U.	I.U.
Yellow corn, ground	35	3.01	1.37	0.70	0.007	0.095	7,000	128,345	
Oats, ground	5	0.60	0.23	0.55	0.005	0.017	2,175	415	
Wheat bran	10	1.69	0.46	0.96	0.014	0.129	5,020	1,330	
Wheat midds	10	1.81	0.48	0.65	0.009	0.093	5,470		
Corn gluten meal	10	4.31	0.20	0.40	0.013	0.038	1,350	166,670	
Soybean meal, 41%	16.5[d]	9.50	1.14	1.23	0.056	0.127	26,875	3,225	
Alfalfa meal, dhy.	5	0.80	0.12	1.35	0.069	0.013	2,310	361,665	
Steamed bone meal	1	0.07	0.03	0.01	0.317	0.150			
Pulverized limestone	1.5				0.570				
Iodized salt	0.5								
Cod liver oil	0.5							408,600	40,860
Choline premix	1.5						34,050		
Riboflavin premix	1.0								
Trace mineral premix	2.5								
Total	100.0	21.79	4.03	5.85	1.060	0.662	84,250	1,070,250	40,860
Recommended[e]		20			1.0	0.6	70,000	200,000	18,000

[a]Data calculated from Morrison, Frank B. (1948) p. 1086-1190, except as indicated. C
[b]Block, Richard J. and Bolling, Diana (1945).
[e]Schweigert, B. S. (1947).
[d]Calculated as 21.5% since 5% soybean meal was added in the premixes.
[e]Recommended allowances as discussed in the Review of Literature.

Table 1. Composition of Basal Ration[a]

Vit. A	Vit. D	Thiamine	Riboflavin	Pantothenic acid	Niacin	Arginine	Histidine	Lysine	Cystine	Methionine	Tryptophan	Ph al
I.U.	I.U.	mg.	mg.	mg.	mg.	%	%	%	%	%	%	
128,345		66.5	17.5	80.5	315.0	0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.095	0.024[b]	0.
415		14.0	2.5	30.0	31.5	0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.014[b]	0.008[b]	0.
1,330		39.0	14.0	136.0	635.0	0.090	0.032	0.056	0.027	0.019	0.024	0.
		58.0	8.0	93.0	443.0	?	?	?	?	?	?	
166,670			60.0	53.0	234.0	0.145	0.100	0.084	0.082	0.100	0.034	0.
3,225		30.1	30.1	133.3	367.7	0.551[b]	0.219[b]	0.551[b]	0.142	0.190[b]	0.152[b]	0.
361,665		10.0	33.5	87.0	100.0	0.037[b]	0.018[b]	0.036[b]	0.018	0.023	0.019	0.
		0.1	0.5	1.0	2.3							
408,600	40,860											
			99.9									
070,250	40,860	217.7	266.0	613.8	2,128.5	0.979	0.453	0.807	0.313	0.441	0.261	1
200,000	18,000	90.0	160.0	500.0	800.0	1.2	0.3	0.9	0.4	0.9 or 0.5	0.25	0

s indicated. Cod liver oil and premixes calculated from Manufacturers' labels.

m[a]

rgin- ine	Histi- dine	Lysine	Cys- tine	Methi- onine	Trypto- phan	Phenyl- alanine	Threo- nine	Leucine	Iso- leucine	Valine	Glycine
%	%	%	%	%	%	%	%	%	%	%	%
0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.095	0.024[b]	0.151[b]	0.109[b]	0.647[b]	0.109[b]	0.139[b]	0.140
0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.014[b]	0.008[b]	0.041[b]	0.021[b]	0.048[b]	0.032[b]	0.039[b]	?
0.090	0.032	0.056	0.027	0.019	0.024	0.053[c]	0.035	0.083[c]	0.054[c]	0.082[c]	?
?	?	?	?	?	?	?	?	?	?	?	?
0.145	0.100	0.084	0.082	0.100	0.034	0.255[c]	0.137[b]	0.841[b]	0.159[b]	0.064[b]	0.170
0.551[b]	0.219[b]	0.551[b]	0.142	0.190[b]	0.152[b]	0.542[b]	0.380[b]	0.627[b]	0.447[b]	0.399[b]	1.634
0.037[b]	0.018[b]	0.036[b]	0.018	0.023	0.019	0.039[c]	0.030	0.061[c]	0.047[c]	0.054[c]	?

9	0.453	0.807	0.313	0.441	0.261	1.081	0.712	2.307	0.848	0.777	1.944
	0.3	0.9	0.4	0.9 or 0.5	0.25	0.9	0.6	1.4	0.6	0.8	1.0

from Manufacturers' labels.

Table 2.

Ingredients	Amount	Protein	Fat	Fiber	Ca	P	Choline	Vit. A	Vit. I
	%	%	%	%	%	%	mg.	I.U.	I.U.
Yellow corn, ground	35	3.01	1.37	0.70	0.007	0.095	7,000	128,345	
Oats, ground	5	0.60	0.23	0.55	0.005	0.017	2,175	415	
Wheat bran	10	1.69	0.46	0.96	0.014	0.129	5,020	1,330	
Wheat midds	10	1.81	0.48	0.65	0.009	0.093	5,470		
Corn gluten meal	10	4.31	0.20	0.40	0.013	0.038	1,350	166,670	
Soybean meal, 41%	13.5[d]	8.18	0.98	1.05	0.048	0.109	23,125	2,775	
Alfalfa meal, dhy.	5	0.80	0.12	1.35	0.069	0.013	2,310	361,665	
Steamed bone meal	1	0.07	0.03	0.01	0.317	0.150			
Pulverized limestone	1.5				0.570				
Iodized salt	0.5								
Cod liver oil	0.5							408,600	40,86
Choline premix	1.5						34,050		
Riboflavin premix	1.0								
Trace mineral premix	2.5								
Liver and glandular meal	3.0	1.99	0.49	0.04	0.019	0.038	13,935		
Total	100.0	22.46	4.36	5.71	1.071	0.682	94,435	1,069,800	40,86
Recommended[e]		20.0			1.06	0.6	70,000	200,000	18,00

[a]Data calculated from Morrison, Frank B. (1948). p. 1086-1190, except as indicated.
[b]Block, Richard J. and Bolling, Diana (1945).
[c]Schweigert, B. S. (1947).
[d]Calculated as 18.5% since 5% soybean meal was added in the premixes.
[e]Recommended allowances as discussed in the Review of Literature.

Table 2. Composition of Liver Meal Ration[a]

e	Vit. A	Vit. D	Thia-mine	Ribo-flavin	Panto-thenic acid	Niacin	Argin-ine	Histi-dine	Lysine	Cys-tine	Methi-onine	Trypto-phan	F a
	I.U.	I.U.	mg.	mg.	mg.	mg.	%	%	%	%	%	%	
)	128,345		66.5	17.5	80.5	315.0	0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.095	0.024[b]	(
)	415		14.0	2.5	30.0	31.5	0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.014[b]	0.008[b]	(
)	1,330		39.0	14.0	136.0	635.0	0.090	0.032	0.056	0.027	0.019	0.024	(
)			58.0	8.0	93.0	443.0	?	?	?	?	?	?	
)	166,670			60.0	53.0	234.0	0.145	0.100	0.084	0.082	0.100	0.034	
5	2,775		25.9	25.9	114.7	316.4	0.474[b]	0.188[b]	0.474[b]	0.122	0.164[b]	0.130[b]	
)	361,665		10.0	33.5	87.0	100.0	0.037	0.018	0.036[b]	0.018	0.023	0.019	
			0.1	0.5	1.0	2.3							
	408,600	40,860											
				99.9									
			0.6	60.6	20.7	240.0	0.117[b]	0.049[b]	0.113[b]	0.025[b]	0.058[b]	0.027[b]	
	,069,800	40,860	214.1	322.4	615.9	2,317.2	1.019	0.471	0.843	0.318	0.473	0.266	
	200,000	18,000	90.0	160.0	500.0	800.0	1.2	0.3	0.9	0.4 or 0.9 0.5		0.25	

t as indicated. Cod liver oil and premixes calculated from manufacturers' labels.

Argin-ine	Histi-dine	Lysine	Cys-tine	Methi-onine	Trypto-phan	Phenyl-alanine	Threo-nine	Leucine	Iso-leucine	Valine	Glycine
%	%	%	%	%	%	%	%	%	%	%	%
0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.095	0.024[b]	0.151[b]	0.109[b]	0.647[b]	0.109[b]	0.139[b]	0.140
0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.014[b]	0.008[b]	0.041[b]	0.021[b]	0.048[b]	0.032[b]	0.039[b]	?
0.090	0.032	0.056	0.027	0.019	0.024	0.053[c]	0.035	0.083[c]	0.054[c]	0.082[c]	?
?	?	?	?	?	?	?	?	?	?	?	?
0.145	0.100	0.084	0.082	0.100	0.034	0.255[c]	0.137[b]	0.841[b]	0.159[b]	0.064[b]	0.170
0.474[b]	0.188[b]	0.474[b]	0.122	0.164[b]	0.130[b]	0.466[b]	0.327[b]	0.540[b]	0.384[b]	0.343[b]	1.406
0.037	0.018	0.036[b]	0.018	0.023	0.019	0.039	0.030	0.061	0.047[c]	0.054[c]	?

117[b]	0.049[b]	0.113[b]	0.025[b]	0.058[b]	0.027[b]	0.131[b]	0.104[b]	0.151[b]	0.101[b]	0.112[b]	0.153[b]
019	0.471	0.843	0.318	0.473	0.266	1.136	0.763	2.371	0.886	0.833	1.869
2	0.3	0.9	0.4 or 0.9 0.5		0.25	0.9	0.6	1.4	0.6	0.8	1.0

ted from manufacturers' labels.

In a communication with Armour and Company, Conquest (1949) stated:

> Our liver and glandular meal contains both hog and beef livers in a varying ratio, depending upon the quantity condemned for food uses and sent to our tanks. It also contains a quantity of lungs, although these are not necessarily condemned lungs, but are from healthy cattle since lungs have no edible market. No vitamins have been extracted from the product. The product goes to drying equipment which we call a dry melter--a horizontal, steam jacketed dryer where the temperature is controlled to about 230-240° at the very end of the drying cycle. In the early stages, as the major portion of the water is driven off, the temperature does not rise above the boiling point of water. This material when dried is simply ground and bagged and sold as liver glandular meal.

A third ration (Table 3) was fed which was identical with the basal ration except 0.5% of the soybean meal was replaced with 0.5% APF supplement No. 3 from Merck and Company, Rahway, New Jersey. The vitamin B_{12} activity as measured by the L.L.D. (Lactobacillus lactis Dorner) method of assay was 12.5 mg. per lb. By including 0.5% of the APF concentrate in the ration, 62.5 μg. of vitamin B_{12} were present in each lb. of ration. The three rations (treatments) were assigned at random to the nine brooders with three groups receiving the same ration. Therefore, by assigning the chicks, groups, and treatments at random to the various brooders (pens), each chick had an equal chance of being chosen to the various treatments, brooders, and groups.

Table

Ingredients	Amount	Protein	Fat	Fiber	Ca	P	Choline	Vit. A.	Vit.
	%	%	%	%	%	%	mg.	I.U.	I.U
Yellow corn, ground	35	3.01	1.37	0.70	0.007	0.095	7,000	128,345	
Oats, ground	5	0.60	0.23	0.55	0.005	0.017	2,175	415	
Wheat bran	10	1.69	0.46	0.96	0.014	0.129	5,020	1,330	
Wheat midds	10	1.81	0.48	0.65	0.009	0.093	5,470		
Corn gluten meal	10	4.31	0.20	0.40	0.013	0.038	1,350	166,670	
Soybean meal, 41%	14[d]	9.28	1.11	1.20	0.057	0.124	26,250	3,150	
Alfalfa meal, dhy.	5	0.80	0.12	1.35	0.069	0.013	2,310	361,665	
Steamed bone meal	1	0.07	0.03	0.01	0.317	0.150			
Pulverized limestone	1.5				0.570				
Iodized salt	0.5								
Cod liver oil	0.5							408,600	40,
Choline premix	1.5						34,050		
Riboflavin premix	1.0								
Trace mineral premix	2.5								
APF premix	2.5								
Total	100.0	21.57	4.00	5.82	1.061	0.659	83,625	1,070,175	40,
Recommended[e]		20			1.0	0.6	70,000	200,000	18,

[a]Data calculated from Morrison, Frank B. (1948). p. 1086-1190, except as indicated.
[b]Block, Richard J. and Bolling, Diana. (1945).
[c]Schweigert, B. S. (1947).
[d]Calculated as 21% since 7% soybean meal was added in the premixes.
[e]Recommended allowances as discussed in the Review of Literature.

Table 3. Composition of APF Ration[a]

P	Choline	Vit. A.	Vit. D	Thiamine	Riboflavin	Pantothenic acid	Niacin	Arginine	Histidine	Lysine	Cystine	Methionine
%	mg.	I.U.	I.U.	mg.	mg.	mg.	mg.	%	%	%	%	%
095	7,000	128,345		66.5	17.5	80.5	315.0	0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.09
017	2,175	415		14.0	2.5	30.0	31.5	0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.01
129	5,020	1,330		39.0	14.0	136.0	635.0	0.090	0.032	0.056	0.027	0.01
093	5,470			58.0	8.0	93.0	443.0	?	?	?	?	?
038	1,350	166,670			60.0	53.0	234.0	0.145	0.100	0.084	0.082	0.10
124	26,250	3,150		29.4	29.4	130.2	359.1	0.538[b]	0.214[b]	0.538[b]	0.139	0.18
013	2,310	361,665		10.0	33.5	87.0	100.0	0.037[b]	0.018[b]	0.036[b]	0.018	0.02
---				0.1	0.5	1.0	2.3					
		408,600	40,860									
	34,050											
					99.9							
	83,625	1,070,175	40,860	217.0	265.3	610.7	2,119.9	0.966	0.448	0.794	0.310	0.4
	70,000	200,000	18,000	90.0	160.0	500.0	800.0	1.2	0.3	0.9	0.4 or 0.5	0.9

190, except as indicated. Cod liver oil and premixes calculated from manufacturers' labels.

premixes.
rature.

Argin-ine	Histi-dine	Lysine	Cys-tine	Methi-onine	Trypto-phan	Phenyl-alanine	Threo-nine	Leucine	Iso-leucine	Valine	Glycine
%	%	%	%	%	%	%	%	%	%	%	%
0.120[b]	0.072[b]	0.060[b]	0.033[b]	0.095	0.024[b]	0.151[b]	0.109[b]	0.647[b]	0.109[b]	0.139[b]	0.140
0.036[b]	0.012[b]	0.020[b]	0.011[b]	0.014[b]	0.008[b]	0.041[b]	0.021[b]	0.048[b]	0.032[b]	0.039[b]	?
0.090	0.032	0.056	0.027	0.019	0.024	0.053[c]	0.035	0.083[c]	0.054[c]	0.082[c]	?
?	?	?	?	?	?	?	?	?	?	?	?
0.145	0.100	0.084	0.082	0.100	0.034	0.255[c]	0.137[b]	0.841[b]	0.159[b]	0.064[b]	0.170
0.538[b]	0.214[b]	0.538[b]	0.139	0.186[b]	0.148[b]	0.529[b]	0.371[b]	0.613[b]	0.436[b]	0.390[b]	1.596
0.037[b]	0.018[b]	0.036[b]	0.018	0.023	0.019	0.039[c]	0.030	0.061[c]	0.047[c]	0.054[c]	?

966	0.448	0.794	0.310	0.437	0.257	1.068	0.703	2.293	0.837	0.768	1.906
2	0.3	0.9	0.4 or	0.9 / 0.5	0.25	0.9	0.6	1.4	0.6	0.8	1.0

ed from manufacturers' labels.

The sources of the various items used in the rations are as follows:

Whole yellow corn, Gilchrist Feed Co., Ames, Iowa

Whole oats, Gilchrist Feed Co., Ames, Iowa

Steamed bone meal, Gilchrist Feed Co., Ames, Iowa

Wheat bran, Nebraska Consolidated Mills Co., Omaha, Neb.

Protein, minimum	14.5%
Fat, minimum	3.0%
Fiber, maximum	12.0%
N.F.E., minimum	51.0%

Wheat midds, Inland Mills, Inc., Des Moines, Iowa

Protein, not less than	15.0%
Fat, not less than	3.5%
Fiber, not more than	7.0%

Corn gluten meal, A. E. Staley Mfg. Co., Decatur, Ill.

Protein, minimum	41%
Fat, minimum	1%
Fiber, maximum	6%
N.F.E., minimum	37%

Soybean meal, Marshall Mills, Inc., Marshalltown, Ia.

Crude protein, not less than	41%
Crude fat, not less than	4%
Crude fiber, not more than	7%
N.F.E., not less than	29%

Alfalfa meal (dhy.), Robert Louks, Laurel, Iowa

Crude protein, minimum	17.0%
Crude fat, minimum	2.5%
Crude fiber, maximum	27.0%
N.F.E., minimum	37.0%

Pulverized limestone, Iowa Limestone Co., Des Moines, Iowa

Calcium carbonate	97.0+%
Elemental calcium	38.8+%
Magnesium	0.34%
Ferric oxide (Fe_2O_3)	0.124%

Morton's iodized salt, Morton Salt Co., Chicago, Ill.

Squibb cod liver oil, E. R. Squibb and Sons, New York
1800 U.S.P. units of vitamin A per gram
180 U.S.P. units of vitamin D per gram

Choline chloride, Merck and Company, Rahway, New Jersey

Riboflavin, Merck and Company, Rahway, New Jersey

Trace mineral premix for poultry, Calcium Carbonate Company, Quincy, Illinois

$MnSO_4$	30%
$FeSO_4$	35%
$CuSO_4$	1.04%
$CoSO_4$	0.635%

The premixes used in the rations were prepared as follows:

Riboflavin premix	
Crystalline riboflavin	1 gm.
Soybean meal	10 lb.
Choline premix	
Choline chloride	0.5 lb.
Soybean meal	9.5 lb.
APF premix	
APF supplement	1.0 lb.
Soybean meal	4.0 lb.
Trace mineral premix	
Trace mineral premix	0.45 lb.
Soybean meal	14.55 lb.

The premixes were first mixed and ground in a mortar and pestle to assure adequate mixing. They were then mixed in a 30-quart bowl of a Hobart mixer No. S-601 made by the Hobart Mfg. Company, Troy, Ohio. The rations were prepared approximately every 10 days. Feed was prepared eight times during the twelve-week period that the experiment was run. The rations were also mixed in a Hobart mixer No. S-601, but a 60-quart bowl was used which mixed 40 to 50

lb. of feed at one time. The mixer was run in the second speed for a period of five minutes to make certain that the feed was mixed properly.

Every two weeks the chicks were weighed individually. Photographs of the average-sized chicks from the groups receiving the same treatment were taken. The feed left in the feeding troughs was weighed at this time in order to determine the feed efficiency (amount of feed eaten per unit of gain). At the end of four weeks the chicks were transferred to "grower" batteries. When the chicks were six weeks of age, they were transferred to "finisher" batteries to give them more head space. In making these transfers the groups of chicks were kept in the same relative positions in the batteries as they were in the brooders. The grower and finisher batteries were manufactured by the MaKomb Steel Products Company, Macomb, Illinois.

At four weeks of age, six chicks were bled every other day; i.e., Monday, Wednesday, and Friday of each week. The bleeding was continued in this manner until the chicks were 12 weeks old. Two chicks from groups receiving the three treatments were bled each time with six different chicks being bled at each bleeding. Thus, in time the entire 90 chicks were bled. The first four chicks of each group, that had been bled previously, were bled again to determine if age produced a change in the blood picture.

When the experiment was completed, a total of 126 blood samples had been studied.

Approximately 2 ml. of blood were obtained directly from the heart by the method described by Hofstad (1950). Each blood sample was placed in a bacteriological test tube which contained 2.4 mg. of dry ammonium oxalate and 1.6 mg. of dry potassium oxalate. This anticoagulant is described by Heller and Paul (1934) and by Wintrobe (1946). A solution of the anticoagulant was prepared by dissolving 1.2 gm. of ammonium oxalate and 0.8 gm. of potassium oxalate in 100 ml. of neutral distilled water. One ml. of 40% formalin was added to prevent deterioration. Two-tenths ml. of this solution was added to each test tube and evaporated to dryness.

The hemoglobin content of the chicken blood was determined by a Klett-Summerson Photoelectric Colorimeter. A stock standard acid hematin solution was prepared after the iron content of a sample of chicken blood (30 ml.) was determined by the method described by Wong (1928). The blood was then diluted with 0.1 N HCl in a 100 ml. volumetric flask so that the stock standard acid hematin solution contained 3% hemoglobin. This stock standard will keep three months in a refrigerator according to Hawk et al. (1947). The nucleated erythrocytes of chicken blood do not give higher readings by the method described

by Wong, whereas in making colorimetric determinations for hemoglobin with an acid hematin solution, the nucleated erythrocytes cause higher readings to be obtained.

Schultze and Elvehjem (1934) overcame the turbidity of acid hematin solutions of chicken blood by first diluting the blood with 0.4% NH_4OH, as Palmer (1918) did with human blood, and then adding a few drops of concentrated HCl. With this method in mind, the unknown blood sample and also the standard acid hematin solution were treated in this manner. It appears that the standard acid hematin solution should be treated with 0.4% NH_4OH as well as the unknown blood sample to overcome the turbidity. Various checks were made on the standard acid hematin solution by using NH_4OH and then acidifying with concentrated HCl and by using 0.1 N HCl without NH_4OH. In all cases the readings were lower on the colorimeter when NH_4OH was used. The standard acid hematin solution was prepared fresh each week by diluting 2.5 ml. of stock standard to 100 ml. with 0.4% NH_4OH and concentrated HCl. NH_4OH (0.4%) was added to the 2.5 ml. of stock standard in a 100 ml. volumetric flask leaving approximately 5 ml. space for the HCl and adjustment of the pH to 1.1. This pH was obtained when the method of Schultze and Elvehjem was followed. The following is a summary of the method used in determining the hemoglobin content of chicken blood:

Place 20 ml. of 0.4% NH_4OH in 50 ml. test tube.

Add 0.1 ml. of blood with blood pipette rinsing three times with the NH_4OH solution.

Stopper test tubes and mix well by inverting three times.

Let stand at least one minute.

Add 0.48 ml. of concentrated HCl or until pH is 1.1.

Stopper test tubes and mix well by inverting three times.

Let stand 40 minutes.

Read unknown and known standard acid hematin solutions in colorimeter.

Calculation: $\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.075 \times \frac{100}{0.1} \times \frac{20.58}{100}$ = gm. of hemoglobin per 100 ml. of blood.

The 0.075 is the gm. per cent hemoglobin in the acid hematin standard (Hawk et al., 1947), and the 0.1 is the amount of blood used. The 20.58 represents the diluted volume of the blood.

The erythrocyte and leucocyte counts were made by Wiseman's method. Olson (1935) found that Wiseman's method was the most satisfactory of the five methods studied for counting erythrocytes. This method was also the better of the two indirect methods for counting leucocytes.

Blood was drawn to the 0.5 mark of a red blood cell

diluting pipette. A diluting fluid consisting of 50 mg. of phloxine, 5 ml. of neutral formalin, and 95 ml. of Ringer's solution was drawn to the 101 mark of the pipette. The blood was diluted 200 times. The method is described by Olson (1948). After adequate mixing of the blood and diluting fluid, the pipettes were stored in a refrigerator for 24 hours. The phloxine stains the erythrocytes a deep pink color and the acidophilic granulocytes (heterophils and eosinophils) are also stained by the phloxine. In counting erythrocytes, 80 of the smallest squares of a bright-line, improved Neubauer hemacytometer are counted. This is done by using the 4 mm. objective and counting the erythrocytes in the four corner squares and one center square of the finely ruled central area of the counting chamber. The total number of cells counted are multiplied by 10,000, if the blood is diluted 1:200, to give the number of erythrocytes per cu. mm. of blood. In counting the stained erythrocytes and acidophilic granulocytes, both counting chambers of a hemacytometer were used. Both counting chambers were filled with diluted blood from one pipette. The counts were made on both sides and averaged. In counting erythrocytes and the acidophilic granulocytes, if the counts varied too much, new counts were made by taking new samples from the blood pipette. The criterion used in judging whether or not the variation between the

two counts was significant was suggested by Nordskog (1949). This test was made by taking the square root of the average of the two counts. If this number was greater than the difference between the counts and the average, the cells counted were considered to be from the same population. In the event the number was less than the difference between the counts and the average, the cells counted were considered to be from different populations and new counts were made from the diluted blood in the pipette. For example, red cell counts of 276 and 236 came from different populations because the square root of their average is 16 and that number is less than 20 which is the difference between the average, 256, and the counts, 276 and 236. On the other hand, counts of 266 and 246 are from the same population and can be expected in the counting of erythrocytes. In nearly all cases that counts were made again, it was found that the new counts were close to one of the first counts which indicated an error in the original counting.

The acidophilic granulocytes were counted in the entire ruled area of the hemacytometer. A differential leucocyte count is necessary in arriving at a total leucocyte count when using Wiseman's indirect method. In calculating the total leucocyte count the following formula (Olson, 1948) was used:

$$\text{Total leucocyte count} = \frac{10}{9}\left[\text{Number of acidophilic cells counted in hemacytometer}\right] \quad 200\ (\text{dilution}) \left[\frac{100}{\text{percentage of acidophilic cells found in blood smear}}\right]$$

A differential leucocyte count was made by staining the blood smear with May-Grünwald and Giemsa combination stain. The staining procedure of the blood smear made from oxalated blood is as follows:

May-Grünwald stain	1 min.
Yellow eosin (water soluble) (Dr. G. Grübler & Co., Leipzig, Germany)	10 sec.
Giema stain	20 min.

In making the differential leucocyte count, 200 cells were counted by the "four-field meander" method recommended by Gradwohl (1943). Marbel's blood-cell calculator was used to record the various cells while making the counts.

The sedimentation rate of the erythrocytes was determined at one-half, one, two, three, and six hour intervals after the Wintrobe hematocrit tubes were filled. The tubes were held in a vertical position in a blood sedimentation rack designed for Wintrobe tubes. The tubes were filled with blood by using Wintrobe filling pipettes. At the end of the six-hour period, the hematocrit tubes were centrifuged for 30 minutes at 3,000 r.p.m. The volume of packed erythrocytes, packed leucocytes and thrombocytes, and plasma expressed in per cent was recorded.

At the end of 12 weeks, 18 chicks were killed for gross and microscopic study of tissues. The smallest, largest, and medium-sized chicks from the three groups receiving the same treatment were killed. Since two chicks from each group and an equal number of males and females were to be killed for tissue study, the first chicks assigned at random in each group meeting these requirements were killed. The chicks were placed in a scale funnel with their heads down. They were killed and bled by decapitation one inch posterior to the occipito-atloid joint.

After the bleeding was completed, a portion of sciatic nerve was taken and later observed between crossed prisms of a polarizing microscope for myelin sheath degeneration. The nerve was fixed in 10% formol-saline solution. On the following day, longitudinal sections, 10μ in thickness, were made of the nerve with a freezing microtome. The sections were floated in water, placed on a clean slide, mounted in glycerin, and covered with a clean cover glass. At this time the sections were examined between crossed prisms of a polarizing microscope. Photomicrographs (X250) were taken of the nerves at the point of greatest birefringence. Panatomic X film was used. Wratten B filter No. 58 and Eastman Kodak X-ray developer gave the best results. The Photo Service Department of Iowa State College made enlargements (X500) of the nerves.

Various tissues were stained with hematoxylin and triosin and by the Marchi method of staining. These tissues were taken at the same time as a portion of the sciatic nerve was taken for study with the polarizing microscope. These tissues consisted of small portions of brain, spinal cord, sciatic nerve, skeletal muscle, heart, liver, kidney, spleen, pancreas, gizzard, proventriculus, small intestine, cecum, rectum, testis or ovary, thyroid, thymus, and lung.

The technique as described by Mallory (1938) was followed for the Marchi method. The tissues were kept in Müller's fluid 18 days. They were then placed in a solution containing two parts of Müller's fluid and one part of a 1% aqueous solution of osmic acid for seven days. Dioxane was used for dehydration. The tissues were embedded in Tissuemat and sectioned 20μ in thickness. Tissuemat is a product manufactured by Fisher Scientific Company.

Tissues stained with hematoxylin and triosin were fixed in Zenker's solution. After 24 hours of fixation, the tissues were dehydrated in the graded alcohols. The tissues were embedded in Tissuemat. Sections, 6μ in thickness, were mounted on gelatinized slides. The sections were stained with hematoxylin and triosin as follows:

1. Xylene #1	5 min.
2. Xylene #2	5 "

3. 95% alcohol #1	3 min.
4. 95% alcohol #2	3 "
5. 70% alcohol	3 "
6. Wash in distilled water	5 "
7. Stain in Delafield's hematoxylin (20 parts of stock solution to 80 parts of sat. ammonium alum sol.)	3 " or until differentiated
8. Wash in distilled water	5 min.
9. Wash in tap water	5 min.
10. Wash in distilled water	2 "
11. 70% alcohol	2 "
12. 90% alcohol	2 "
13. Stain in triosin (0.5% in 90% alcohol)	10 sec. or until differentiated
14. 95% alcohol	1 min.
15. Absolute alcohol #1	3 "
16. Absolute alcohol #2	3 "
17. Xylene #1	5 "
18. Xylene #2	5 "

19. Mount in Permount (Fisher Scientific Co.)

Photomicrographs were taken of certain tissues stained by the Marchi method and also by hematoxylin and triosin. Panatomic X film was used with Wratten B filter No. 58. Kodak Universal M-Q Developer was used for film and paper development. All of the pictures were printed on Ad-type paper made by Eastman Kodak Company except those taken of the sciatic nerves using the polarizing microscope.

IV. RESULTS

A. Growth Data

During the first two weeks of the experiment one or two chicks in each group showed some "pasting up". It did not become serious and no losses occurred. Ten days after the experiment was begun, two chicks (Tables 9 and 11), 109H receiving the liver meal ration and 101I receiving the APF ration, developed perosis. In three weeks' time 104J receiving the liver meal (Table 8) showed signs of perosis. Two chickens, 104I and 106K, receiving the basal ration (Tables 5 and 4) became affected with perosis in six and seven weeks, respectively. A mild case of perosis was present in chick 102I (Table 10) receiving the APF ration at 10 weeks. The chicks with perosis remained with the other chicks throughout the experiment. Feed and water were readily available which accounted for their gain along with the other chicks. No doubt their growth was retarded to a certain extent. All chicks affected with perosis were males.

Tables 4 through 12 show the weight gains of the chicks at two- week intervals. The gains in weight were placed in the tables since these figures were used in the

Table 4. Weight Gains of Chicks Receiving Basal Ration in Pen 1

Chick no.	Sex	Start-ing wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
101D	M	42	60	235	470	816	1213	1667
101G	F	47	62	270	541	895	1237	1545
101K*	M	43	60	315	624	1038	1563	1988
104H	F	46	85	252	480	791	1109	1384
103L*	F	44	58	234	458	766	1100	1449
105J	F	44	42	130	265	556	876	1219
108C	M	38	68	261	471	922	1253	1636
109D	M	47	75	288	432	819	1201	1587
106K**	M	43	74	315	502	902	1180	1394
108K	F	42	42	166	372	663	971	1266
	Avg.	43.6	62.6	246.6	461.5	821.8	1170.3	1513.5

*Tissues taken for histologic study
**Developed perosis at 7 wk.

Table 5. Weight Gains of Chicks Receiving Basal Ration in Pen 2

Chick no.	Sex	Start-ing wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
101J*	F	42	65	213	462	734	1002	1293
104I**	M	45	66	243	499	780	1108	1395
102G*	M	46	64	252	512	900	1324	1716
105C	M	47	77	266	505	871	1304	1725
104L	F	43	50	219	433	746	1093	1354
107H	M	42	74	275	527	952	1394	1838
106E	M	37	48	268	560	1002	1458	1923
108H	M	39	51	265	509	936	1375	1812
108L	F	42	71	217	416	675	956	1187
109K	F	42	24	154	394	687	990	1279
Avg.		42.5	59.0	236.2	481.7	828.3	1200.4	1552.2

*Tissues taken for histologic study
**Developed perosis at 6 wk.

Table 6. Weight Gains of Chicks Receiving Basal Ration in Pen 3

Chick no.	Sex	Start-ing wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
102C	F	37	95	264	547	892	1239	1546
102E	F	42	72	182	259	466	726	904
104D	F	36	67	170	370	662	944	1207
104E*	M	45	59	141	326	639	1061	1549
106H	F	38	75	254	484	771	1093	1331
108I	M	39	64	143	318	675	1069	1482
106F	M	37	31	91	184	406	793	1201
109J	M	45	47	114	287	625	1085	1556
109L	M	40	58	142	268	571	957	1370
109G*	F	39	18	63	148	317	530	791
Avg.		39.8	58.6	156.4	319.1	602.4	949.7	1293.7

*Tissues taken for histologic study

Table 7. Weight Gains of Chicks Receiving Liver Meal Ration in Pen 4

Chick no.	Sex	Start-ing wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
101F*	F	45	64	241	452	724	1003	1311
103I	F	45	83	247	466	759	1037	1305
105I	F	40	80	258	536	893	1214	1512
103G	F	47	104	316	574	967	1248	1500
105H	F	47	91	307	544	937	1314	1670
105E*	M	40	67	278	485	929	1390	1800
106J	M	40	75	283	532	895	1154	1135**
107L	M	45	113	324	533	999	1442	1805
108F	M	45	36	121	256	590	1026	1413
106G	M	46	80	253	534	940	1376	1827
	Avg.	44.0	79.3	262.8	491.2	863.3	1220.4	1571.4

*Tissues taken for histologic study

**Chick had lost weight the last two weeks due to embryonal nephroma which will be described later. Chick gain was not included in average.

Table 8. Weight Gains of Chicks Receiving Liver Meal Ration in Pen 5

Chick no.	Sex	Starting wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
102F*	M	42	128	308	529	1030	1523	1996
102J*	F	43	61	242	479	792	1061	1272
102H	F	38	83	250	459	806	1167	1467
104C	F	44	125	309	573	872	1222	1486
104J**	M	43	85	311	514	962	1241	1345
106L	F	44	57	259	526	802	1078	1327
107J	F	40	102	330	583	938	1269	1531
107F	F	40	70	236	526	862	1212	1527
107K	F	44	106	300	491	891	1186	1425
108E	F	45	95	335	574	1002	1342	1619
	Avg.	42.3	91.2	288.0	525.4	895.7	1230.1	1499.5

*Tissues taken for histologic study
**Developed perosis in three weeks

Table 9. Weight Gains of Chicks Receiving Liver Meal Ration in Pen 6

Chick no.	Sex	Start-ing wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
103D	F	39	60	246	498	827	1134	1407
101E	F	43	100	316	590	884	1178	1413
103H	M	39	102	313	546	1111	1603	1991
104F*	M	41	98	319	599	1107	1581	2055
107C	F	46	72	301	579	979	1348	1689
108D	M	40	67	178	278	612	997	1391
105F	M	38	95	304	450	943	1342	1782
105K	F	42	55	194	409	687	996	1294
109H**	M	45	99	298	558	937	1190	1364
109E*	F	45	80	303	540	967	1315	1603
	Avg.	41.8	82.8	277.2	504.7	905.4	1268.4	1598.9

*Tissues taken for histologic study
**Developed perosis in 10 days

Table 10. Weight Gains of Chicks Receiving APF Ration in Pen 7

Chick no.	Sex	Starting wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
101C*	M	44	80	287	518	974	1355	1723
102I**	M	39	60	209	477	829	1134	1415
102L*	F	38	114	313	568	875	1173	1401
103C	M	39	80	270	553	893	1331	1766
106D	M	40	82	285	536	1042	1500	1950
105L	F	41	48	191	414	707	1001	1290
102K	M	38	44	179	403	732	1121	1577
104K	F	46	95	284	506	816	1135	1416
104G	F	37	60	222	469***			
109I	F	43	78	268	489	825	1144	1396
	Avg.	40.5	74.1	250.8	493.3	854.8	1210.4	1548.2

*Tissues taken for histologic study

**Developed perosis in 10 weeks

***Chick died shortly after blood sample was taken the day before the six-week weighing. Chick gain was included in average.

Table 11. Weight Gains of Chicks Receiving APF Ration in Pen 8

Chick no.	Sex	Starting wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
101I**	M	47	107	281	515	731	1025	1341
103F*	M	44	63	219	448	795	1141	1454
101H	M	37	105	296	558	984	1317	1594
103E	M	44	39	158	358	642	979	1336
103J	F	40	109	291	519	820	1104	1376
107D	F	47	76	274	564	885	1211	1521
109C	M	37	102	290	506	857	1206	1482
108J*	F	37	41	192	430	697	959	1195
109F	F	47	111	307	512	810	1073	1328
107G	M	45	49	194	463	824	1282	1646
	Avg.	42.5	80.2	250.2	487.3	804.5	1129.7	1427.3

*Tissues taken for histologic study
**Developed perosis in 10 days

Table 12. Weight Gains of Chicks Receiving APF Ration in Pen 9

Chick no.	Sex	Starting wt.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
102D	F	37	85	255	481	762	1085	1302
101L*	F	44	63	206	432	757	1074	1546
106I*	M	39	69	305	538	1101	1622	2065
105D	M	47	73	225	457	777	1151	1527
106C	F	39	54	232	456	745	1020	1260
107I	M	43	75	239	484	872	1313	1726
103K	M	43	99	319	499	983	1368	1763
107E	F	37	94	310	568	957	1297	1586
105G	M	47	55	205	461	866	1303	1747
108G	M	46	117	353	461	1037	1519	2005
	Avg.	42.2	78.4	264.9	483.7	885.7	1275.2	1652.7

*Tissues taken for histologic study

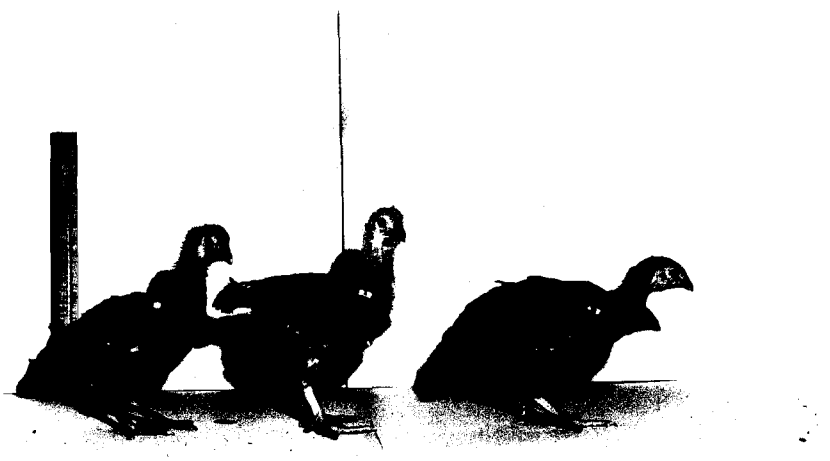
statistical analyses. By adding the gain in weight to the starting weight, the chick's weight can be obtained.

A summary of the weight gains is found in Table 13.

Table 13. Average Weight Gain per Pen of Chicks Receiving the Basal, Liver Meal, and APF Rations

Pen	Ration	Avg. starting wt	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Basal	43.6	62.6	246.6	461.5	821.8	1170.3	1513.5
2	"	42.5	59.0	236.2	481.7	828.3	1200.4	1552.2
3	"	39.8	58.6	156.4	319.1	602.4	949.7	1293.7
	Avg.	41.97	60.07	213.1	420.8	750.8	1106.8	1453.1
4	Liver meal	44.0	79.3	262.8	491.2	863.3	1220.4	1571.4
5	"	42.3	91.2	288.0	525.4	895.7	1230.1	1499.5
6	"	41.8	82.8	277.2	504.7	905.4	1268.4	1598.9
	Avg.	42.7	84.43	276.0	507.1	888.1	1239.6	1556.6
7	APF	40.5	74.1	250.8	493.3	854.8	1210.4	1548.2
8	"	42.5	80.2	250.2	487.3	804.5	1129.7	1427.3
9	"	42.2	78.4	264.9	483.7	885.7	1275.2	1652.7
	Avg.	41.73	77.57	255.3	488.1	848.3	1205.1	1542.7

Figures 1 through 5 are gross pictures of average-weight chicks receiving the three rations. The chicks that were photographed had gained approximately the same as those receiving the same ration. It might be difficult to detect much difference in size among the chicks in each picture; however, the weights in Table 13 and these weights plotted in Figure 6 show a slight difference between the chicks



...re 1. Chicks, four weeks of age, receiving the basal ...ft), liver meal (center), and APF (right) rations.

Left - 101J, 313 gm. gain (315.1 avg.)
Center - 105[illegible], 273 gm. gain (276.0 avg.)
Right - 102D, 355 gm. gain (355.5 avg.)

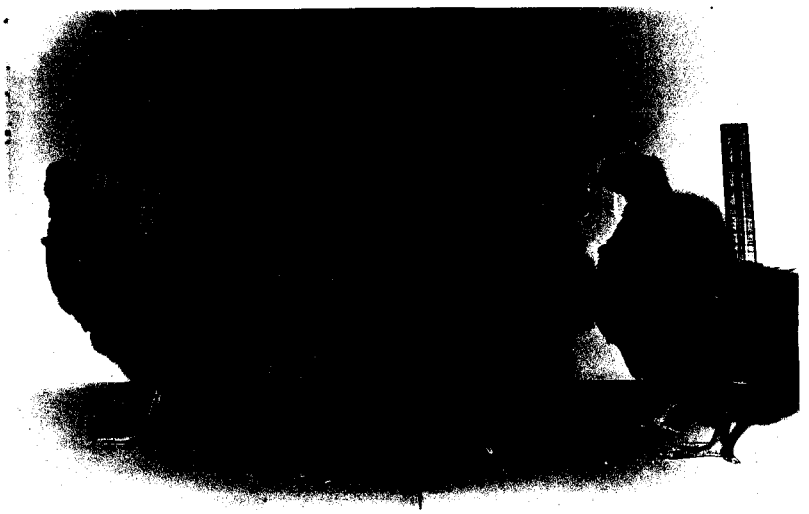


Figure 2. Chicks, six weeks of age, receiving the basal (left), liver meal (center), and ADF (right) rations.

Left - 108L, 416 gm. gain, (420.8 avg.)
Center - 107L, 401 gm. gain, (407.1 avg.)
Right - 102L, 428 gm. gain, (428.1 avg.)

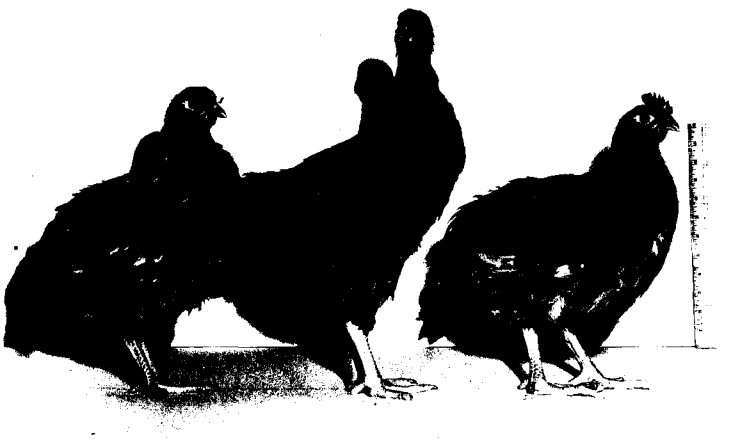


Figure 3. Chicks, eight weeks of age, receiving the basal (left), liver meal (center), and AFF (right) rations.

Left - 104L, 746 gm. gain, (750.8 avg.)
Center - 101M, 884 gm. gain, (888.1 avg.)
Right - 109C, 857 gm. gain, (848.5 avg.)

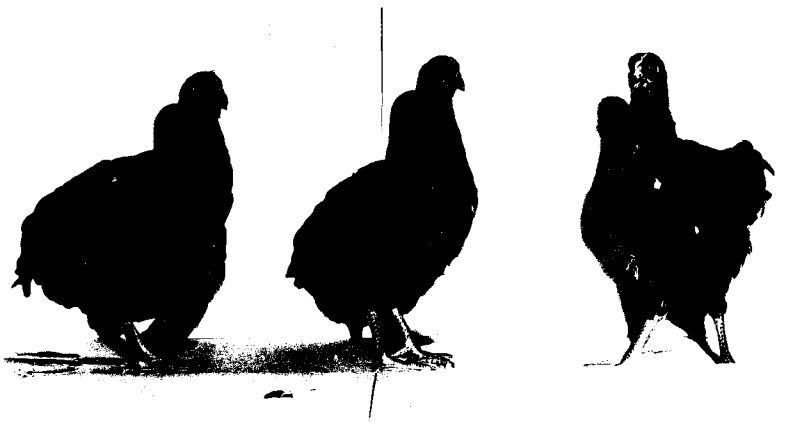


Figure 4. Chicks, 10 weeks of age, receiving the basal (left), liver meal (center), and MP (right) rations.

Left - 1041, 1109 gm. gain, (1106.8 avg.)
Center - 1046, 1[illegible] gm. gain, (1[illegible] avg.)
Right - 10[illegible], 1[illegible] gm. gain, (1[illegible] avg.)

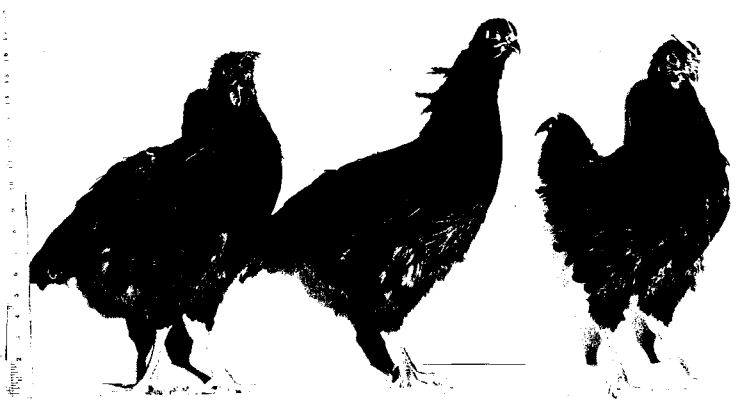


Figure 5. Chicks, 12 weeks of age, receiving the basal (left), liver meal (center), and AFT (right) rations.

Left - 100I, 14[illegible] gm. gain, (1[illegible]0.1 avg.)
Center - 109 , 1303 gm. gain, (1[illegible] avg.)
Right - 10[illegible], 1577 gm. gain, (1[illegible] avg.)

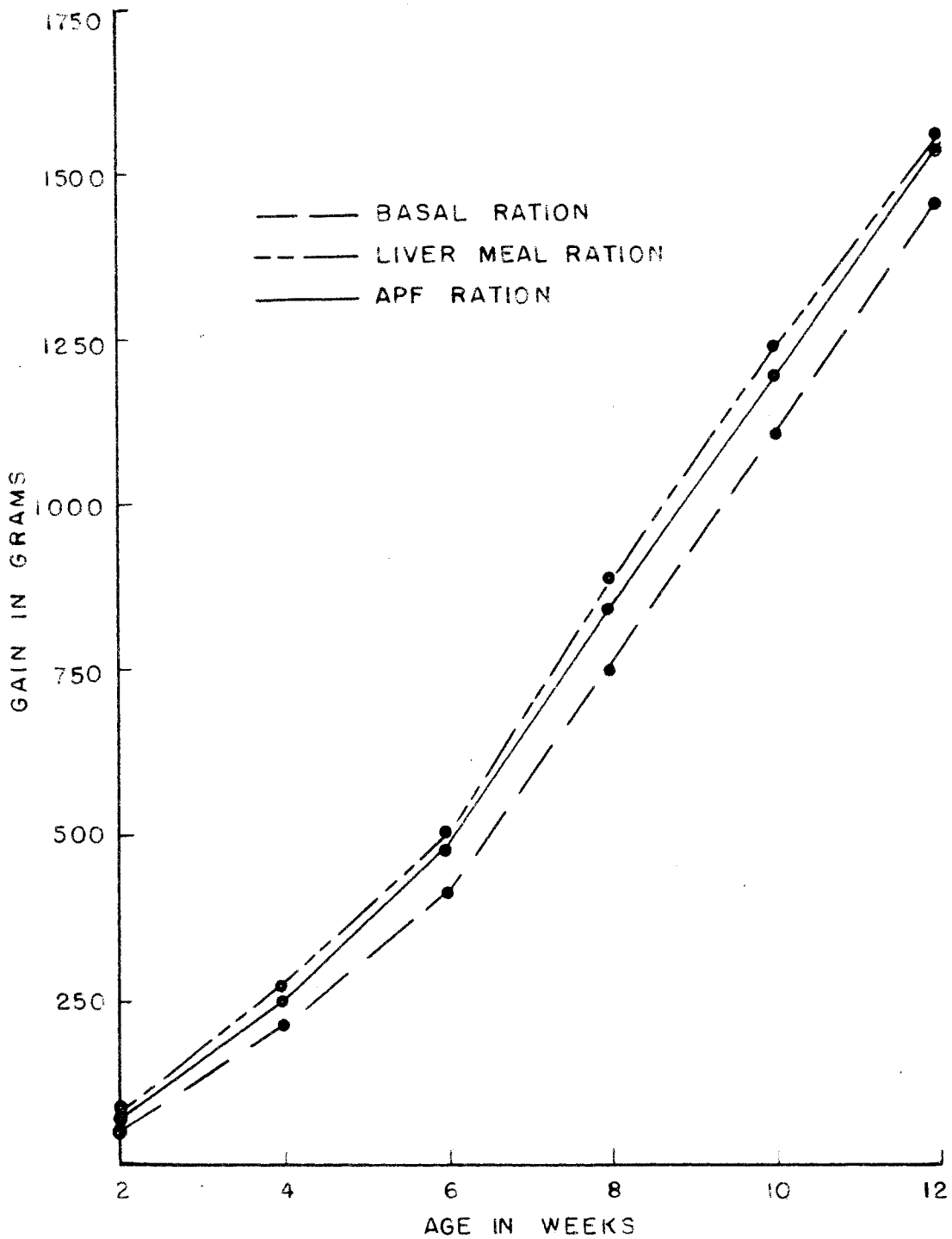


Figure 6. Average weight gains of male and female chicks receiving the basal, liver meal, and APF rations.

receiving the liver meal ration and the APF ration. The chicks receiving the basal ration did not gain as rapidly as the other chicks. The chicks receiving the basal ration and APF ration showed a leg weakness, assuming a "squatting" position in many cases. This condition is noticeable in some of the pictures. At the end of the experiment most of the chicks fed the APF ration had overcome this weakness.

No sex distinction was made during the course of the experiment. Males and females were present in the nine pens. After the experiment was completed, the data were separated according to sex. It was assumed that males and females responded differently to the treatments. Table 14 shows the arithmetic mean ($\bar{x}$) and standard deviation or error of the mean ($s_{\bar{x}}$) of the weight gains of male and female chicks receiving the three rations. It is obvious that the standard error of the mean was larger for the males than the females. This indicates that the deviation from the mean was consistently greater for the males than the females. The standard error of the mean is obtained by summing the squares of each deviation from the mean, dividing the number by the degrees of freedom (n-1), and extracting the square root of that number. For example, the standard error of the mean for two-week-old male chicks was obtained by subtracting 7,643, the sum of

Table 14. The Mean and Standard Error of the Mean of Weight Gains from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

Age in weeks	Sex	Basal $\bar{X}$ and $s_{\bar{x}}$	Liver meal $\bar{x}$ and $s_{\bar{x}}$	APF $\bar{x}$ and $s_{\bar{x}}$
2	M	61.0 ± 5.39	87.08 ± 6.22	76.41 ± 5.23
2	F	59.0 ± 3.80	82.67 ± 3.35	79.08 ± 3.94
4	M	225.88 ± 29.38	274.17 ± 33.92	253.77 ± 28.50
4	F	199.14 ± 5.29	277.22 ± 4.66	257.31 ± 5.49
6	M	437.13 ± 44.94	484.50 ± 51.90	484.41 ± 43.60
6	F	402.07 ± 13.04	522.17 ± 11.50	492.92 ± 13.53
8	M	806.50 ± 68.02	921.25 ± 78.54	878.65 ± 65.99
8	F	687.21 ± 20.55	866.06 ± 18.12	804.67 ± 22.19
10	M	1208.63 ± 72.94	1322.08 ± 84.22	1274.53 ± 70.76
10	F	990.29 ± 27.91	1184.67 ± 24.61	1106.33 ± 30.14
12	M	1614.94 ± 79.02	1706.27 ± 95.30	1635.94 ± 76.66
12	F	1268.21 ± 39.28	1464.44 ± 34.64	1384.75 ± 42.43

squares for subclasses, pens (calculated from Table 15), from the total sum of squares, 22,425.78. This gave 14,782.79, the sum of squares for individuals. The treatment sum of squares was 4,855. The difference between subclass sum of squares and treatment sum of squares is 2,788, which is the sum of squares for cages treated alike. The degrees of freedom for cages treated alike are six, so 2,788 divided

by six gives 464.7. This number is the variance of the pens treated alike. The standard error of the mean, 5.39 (Table 14), for two-week-old male chicks was obtained by dividing 464.7 by 16, the number of males fed the basal ration, and then by extracting the square root of that number.

Figures 7 and 8 demonstrate the average weight gains of the male and female chicks receiving the basal, liver meal, and APF rations. There is a greater difference among the female chicks receiving the three rations than the males. At six weeks of age, the males fed the liver meal and APF rations had made the same average gain.

The average weight gains of male and female chicks receiving basal, liver meal, and APF rations are presented in Figures 9, 10, and 11, respectively. The female chicks fed the basal ration (Figure 9) did not gain as rapidly as the males throughout the experiment, whereas the female chicks receiving the liver meal and APF rations (Figures 10 and 11) gained as rapidly as the males for six or seven weeks.

In order to determine whether or not the liver meal or APF concentrate produced a significant growth response in the chicks, an analysis of variance was run on the weight gains obtained from male and female chicks at the various weighings. Table 15 shows the weight gains of two-week-old males receiving the three rations. Unequal subsample

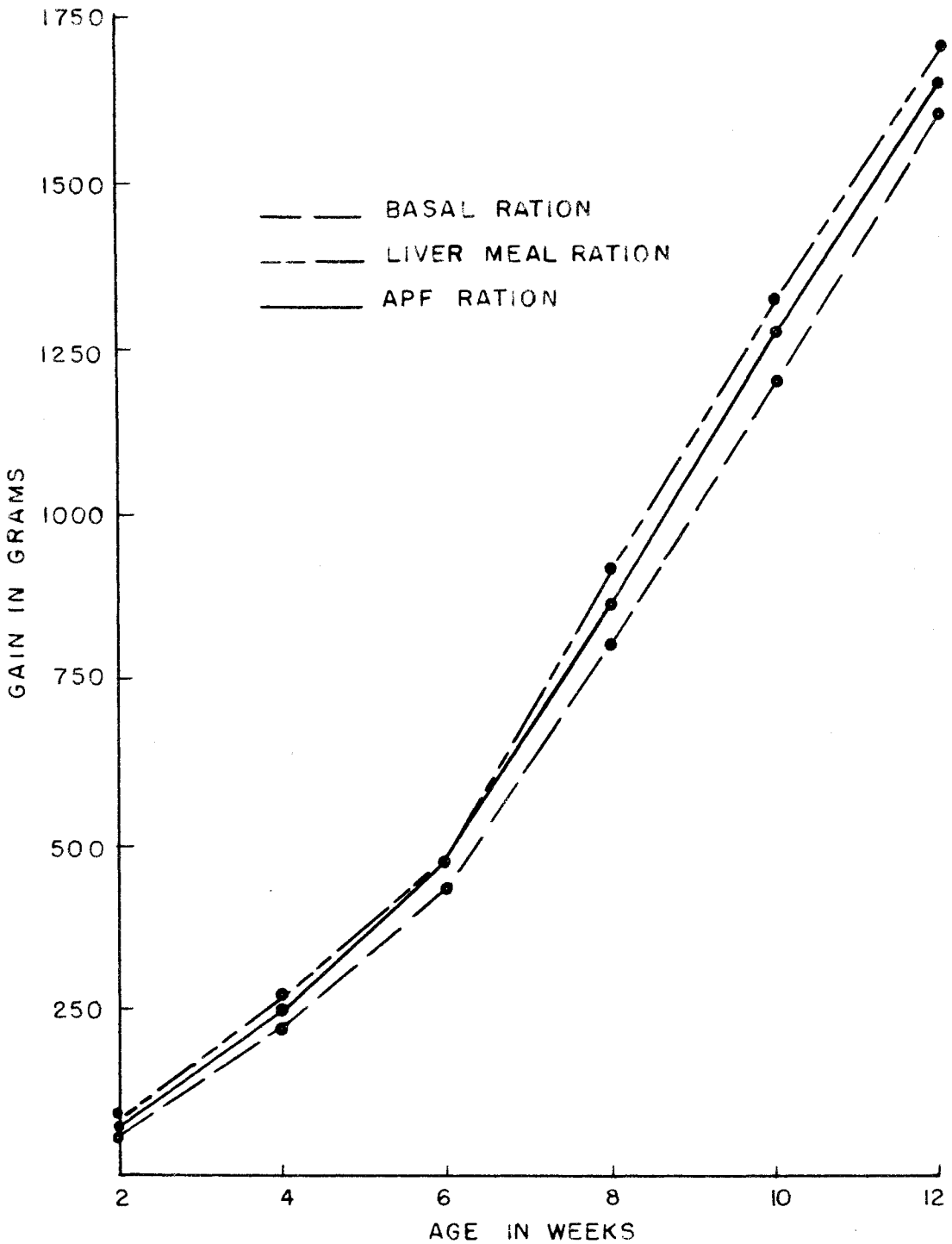


Figure 7. Average weight gains of male chicks receiving the basal, liver meal, and APF rations.

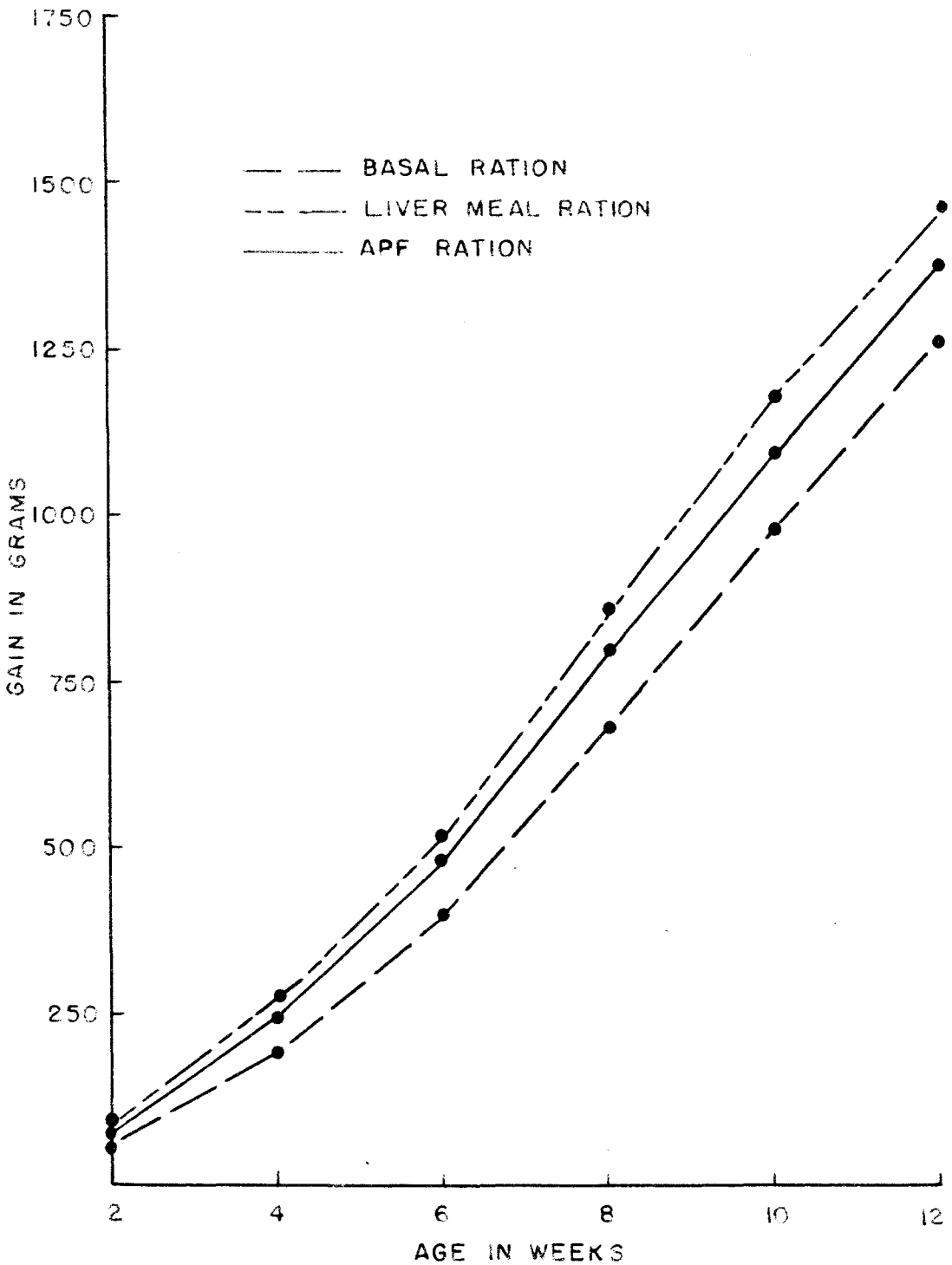


Figure 8. Average weight gains of female chicks receiving the basal, liver meal, and APF rations.

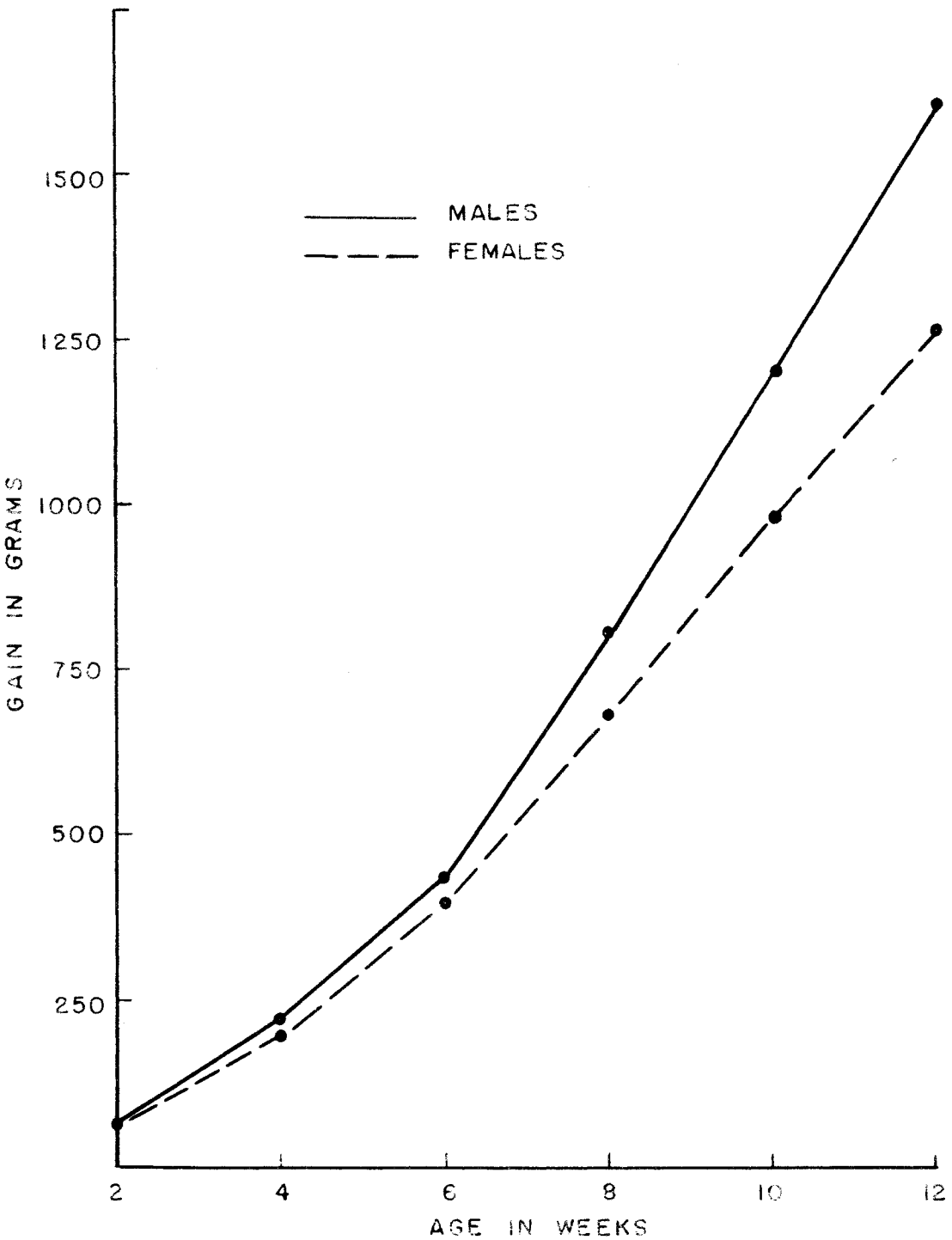


Figure 9. Average weight gains of male and female chicks receiving the basal ration.

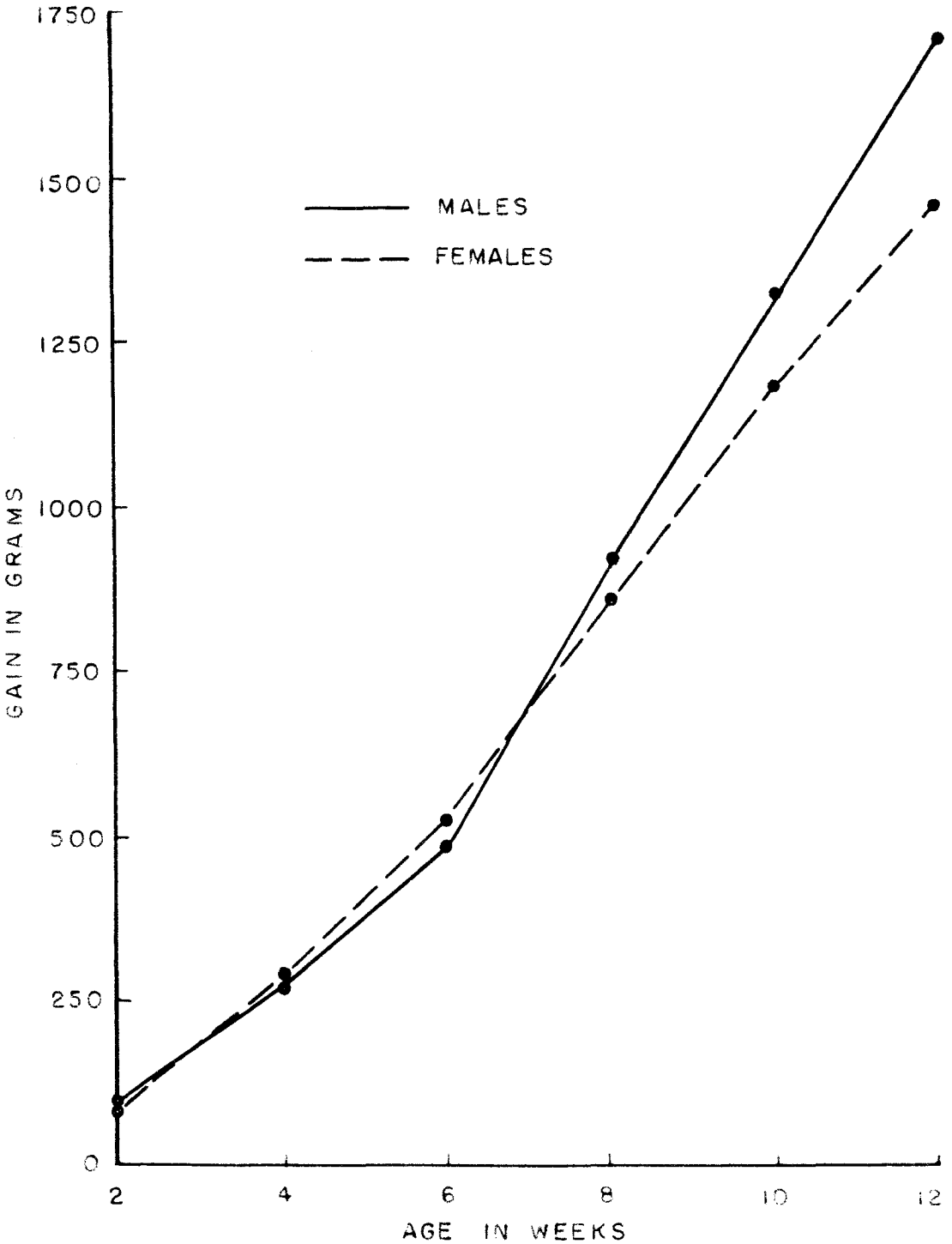


Figure 10. Average weight gains of male and female chicks receiving the liver meal ration.

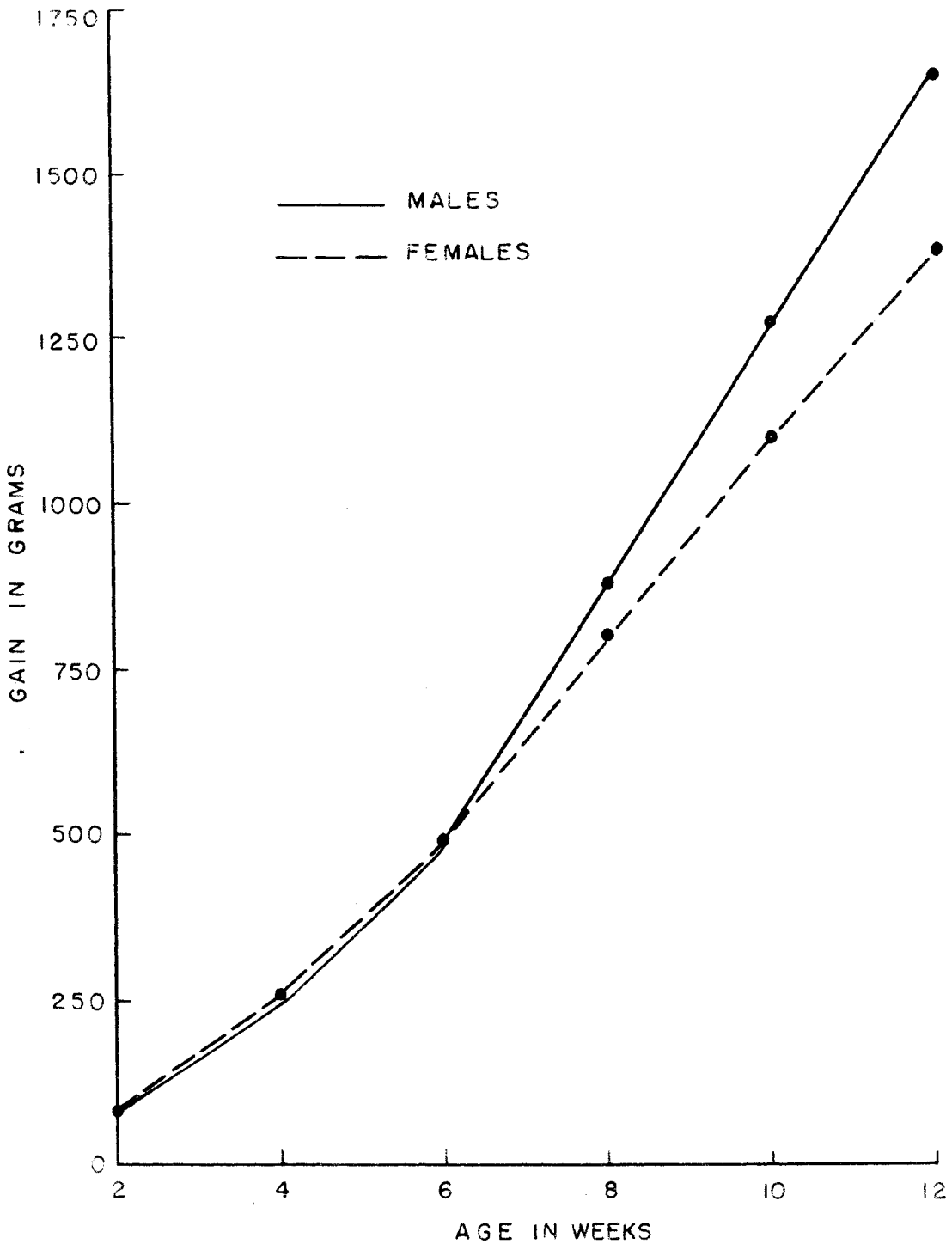


Figure 11. Average weight gains of male and female chicks receiving the APF ration.

Table 15. Weight Gains of Two-week-old Male Chicks Receiving the Basal, Liver Meal, and APF Rations

	Pens fed basal			Pens fed liver meal			Pens fed APF			
	1	2	3	4	5	6	7	8	9	Total
	60	66	59	67	128	102	80	107	69	
	60	64	64	75	85	98	60	63	73	
	68	77	31	113		67	80	105	75	
	75	74	47	36		95	82	39	99	
	74	48	58	80		99	44	102	55	
		51						49	117	
SX	337	380	259	371	213	461	346	465	488	3,320
k	5	6	5	5	2	5	5	6	6	45

X = individual observations
SX = sum of individual observations
k = items per subsample

numbers, as in Table 15, have to be handled differently than equal subsample numbers. The total sum of squares, 22,425.78, is obtained by totaling the square of each observation, 267,368, and subtracting that number from the correction for sum of squares, $\frac{3,320^2}{45}$, or 244,942.22. The subclasses (pens) sum of squares are calculated by squaring each subclass, dividing each square by the number of observations in each subclass, totaling the quotients, and subtracting that total from the correction term $\left(\frac{337^2}{5} + \frac{380^2}{6} + \ldots + \frac{488^2}{6} - \frac{3,320^2}{45} = 7,643\right)$. The treatment sum of squares is obtained by totaling the three subclasses in each treatment, squaring these numbers, dividing by the

number of observations per treatment, totaling the quotients, and subtracting the total from the correction term $\left(\frac{(337 + 380 + 259)^2}{16} + \frac{(371 + 213 + 461)^2}{12} + \frac{(346 + 465 + 488)^2}{17} - \frac{3,320^2}{45} = 4,855\right)$.

The following shows the completed analysis of variance of weight gains for the two-week-old males with a preliminary analysis of variance and also of pen means:

Preliminary Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	44	22,425.78	
Pens	8	7,643	
Individuals	36	14,782.78	410.6

Analysis of Variance of Pen Means

S.V.	D.F.	S.S.	M.S.
Pens	8	7,643	
Treatments	2	4,855	2,427.5
Pens treated alike	6	2,788	464.7

Completed Analysis of Variance for Two-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	4,855	2,427.5*
Pens treated alike	6	2,788	464.7
Individuals	36	14,782.78	410.6
Total	44	22,425.78	

*Significant at 5% level

By dividing the treatments' mean square by the mean square for pens treated alike, a test of significance (F-test) is performed to see if the treatments (rations) affected the weight gains. This is done by dividing 2,427.5 by 464.7. The quotient is 5.22 which is slightly larger than the 5% level (Snedecor, 1946) but smaller than the 1% level of significance. Since $F_{.05} = 5.14$ with 2 and 6 d.f., there is a significant difference among weight gains caused by treatments. If the F-value had been larger than the 1% level (10.92), the treatments would have produced a highly significant difference. Another F-test may be performed by dividing 464.7 by 410.6 to see if environmental effects have appeared. Since the F-value is not significant, the environmental conditions were apparently satisfactory. If environmental effects had been present, a large mean square for pens treated alike could be expected. Thus, it is necessary to keep out environmental effects to make an accurate test for treatment effects.

The question may arise between what treatments does this significant difference occur. The least significance difference (L.S.D.) that may exist between the treatment means before a significant level is reached will determine what treatments were effective in producing a change in the weight gains. The L.S.D. will be discussed later.

The following is a series of completed analyses of

variance of weight gains for male and female chicks at the various weighings:

Completed Analysis of Variance for Two-week-old-Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	4,834	2,417**
Pens treated alike	6	1,213	202
Individuals	36	19,058	529.4
Total	44	25,105	

**Significant at 1% level

Completed Analysis of Variance for Four-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	16,538.5	8,269.25
Pens treated alike	6	82,862.5	13,810.4**
Individuals	36	89,408	2,483.6
Total	44	188,809.0	

Completed Analysis of Variance for Four-week-old Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	49,885.4	24,942.7**
Pens treated alike	6	2,347.6	391.3
Individuals	36	93,458	2,596.1
Total	44	145,691.0	

Completed Analysis of Variance for Six-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	23,092	11,548
Pens treated alike	6	193,912	32,318.67**
Individuals	36	204,549	5,682
Total	44	421,553	

Completed Analysis of Variance for
Six-week-old Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	118,598	59,299**
Pens treated alike	6	14,288	2,381.33
Individuals	36	234,812	6,522.6
Total	44	367,698	

Completed Analysis of Variance for
Eight-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	95,872	47,936
Pens treated alike	6	444,131	74,021.8**
Individuals	36	594,409	16,511.4
Total	44	1,134,412	

Completed Analysis of Variance for
Eight-week-old Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	254,355	127,177.5**
Pens treated alike	6	35,460	5,910
Individuals	35	490,042	14,001.2
Total	43	779,857	

Completed Analysis of Variance for
Ten-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	91,429	45,714.5
Pens treated alike	6	510,729	85,121.5*
Individuals	36	1,005,946	27,942.9
Total	44	1,608,104	

Completed Analysis of Variance for
Ten-week-old Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	297,941	148,970.5**
Pens treated alike	6	65,410	10,901.7
Individuals	35	718,812	20,537.5
Total	43	1,082,163	

Completed Analysis of Variance for
12-week-old Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	54,412	27,206
Pens treated alike	6	599,364	99,894
Individuals	35	1,636,400	46,754
Total	43	2,290,176	

Completed Analysis of Variance for
12-week-old Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	303,569	151,784.5**
Pens treated alike	6	129,596	21,599.3
Individuals	35	901,793	25,762
Total	43	1,334,958	

Figures 12 and 13 show the F-values for treatment effects and environmental effects, respectively. The treatments (rations) produced a highly significant difference (Figure 12) in the weight gains of the female chicks for the first 10 weeks and a significant difference at 12 weeks. At two weeks of age, the weight gains for the male chicks were significant; however, the treatments had no effect on weight gains for the remaining 10 weeks. Figure 13 may explain the difference between male and female chicks as observed in Figure 12. The environmental effects (Figure 13) were highly significant for males at four, six, and eight weeks of age and significant at 10 weeks. The F-values for environmental effects for females did not approach the significant level (5%).

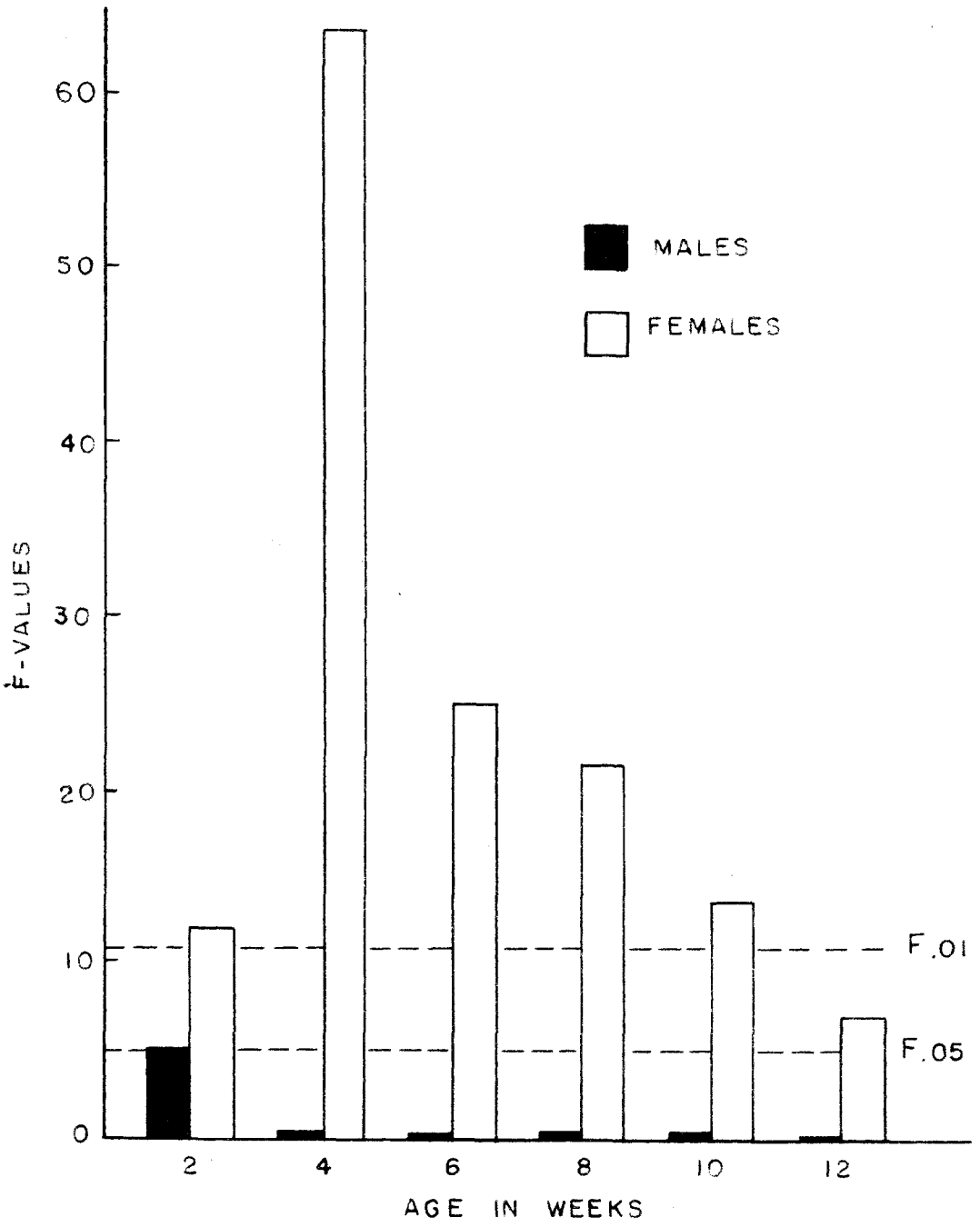


Figure 12. F-values showing treatment effects in the weight gains of male and female chicks receiving the basal, liver meal, and APF rations.

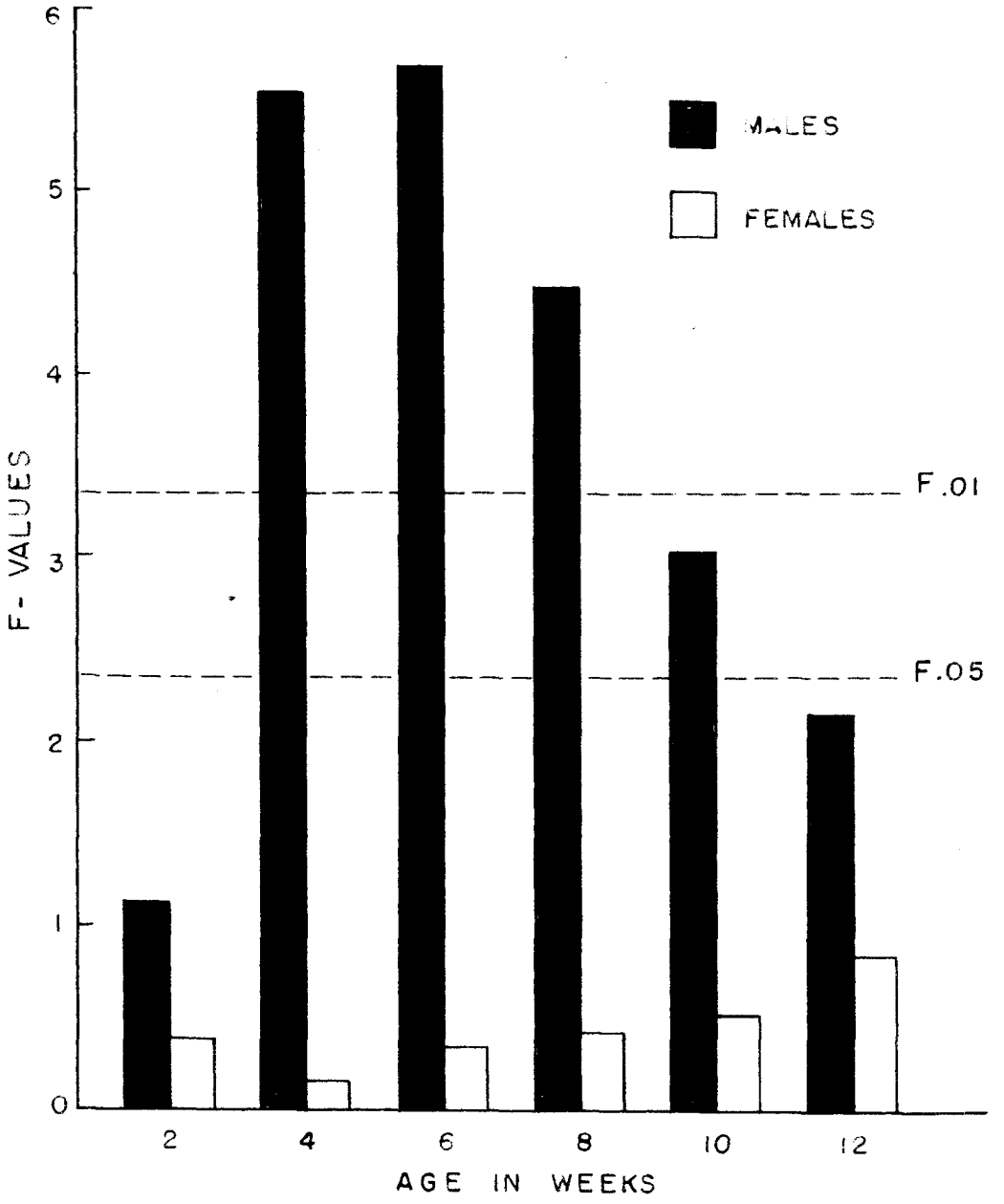


Figure 13. F-values showing environmental effects in the weight gains of male and female chicks receiving the basal, liver meal, and APF rations.

The least significant differences that can exist between the average weight gains of chicks receiving the three rations are shown in Table 16. Each difference is obtained by subtracting the larger average gain of chicks fed one ration from the average gain of the chicks fed a second ration. The L.S.D. is calculated for all comparisons at the 5% level and at the 1% level of those being significant at 5%. The L.S.D. at the 5% level (15.69) between weight gains of two-week-old male chicks fed basal and liver meal rations is derived by extracting the square root of the sums of the quotients of the mean square for individuals divided by the number of observations in each treatment and then multiplying this number by the t-value at 36 d.f. $\left(\text{L.S.D.} = 2.028\sqrt{\frac{410.6}{16} + \frac{410.6}{12}}\right)$. Table 16 shows that the weight gains of female chicks receiving liver meal and APF rations are different either at the 1 or 5% levels, when compared with the gains of female chicks receiving the basal ration with the exception of those fed the APF ration at 12 weeks of age. The average gains of male chicks receiving liver meal and APF rations were different statistically when compared with those fed the basal ration at two, four, and eight weeks of age for the liver meal ration and only at two weeks of age for the APF ration. The differences in weight gains of chicks receiving liver meal and APF rations were not significant when analyzed statistically.

Table 16. Least Significant Differences Between Average Weight Gains of Chicks Receiving the Basal, Liver Meal, and APF Rations Using the t-values at 1 and 5% Levels

Age in weeks	Sex	Basal and liver meal			Basal and APF			Liver meal and APF	
		Difference	L.S.D. 5%	L.S.D. 1%	Difference	L.S.D. 5%	L.S.D. 1%	Difference	L.S.D. 5%
2	M	26.08**	15.69	21.05	15.41*	14.31	19.20	10.67	15.49
2	F	23.67**	16.63	22.30	20.08*	17.97	24.10	3.59	16.98
4	M	48.29*	38.60	51.76	27.89	35.20		20.40	38.11
4	F	78.08**	36.82	49.39	58.17**	39.79	53.38	19.91	37.61
6	M	47.37	58.38		47.28	53.25		0.09	57.64
6	F	120.10**	58.37	78.28	90.85**	63.09	84.61	29.25	59.62
8	M	114.75*	99.52	133.47	72.15	90.77		42.60	98.25
8	F	178.85**	85.60	114.87	117.46*	94.49	126.80	61.39	89.52
10	M	113.45	129.46		65.90	118.08		47.55	127.82
10	F	194.38**	103.67	139.11	116.04*	114.45	153.57	78.34	108.42
12	M	91.33	171.92		39.00	152.89		52.33	169.85
12	F	196.23**	116.11	155.80	116.54	128.18		79.69	121.43

*Significant at 5% level
**Significant at 1% level

B. Feed Efficiency

The feed efficiency, amount of feed consumed per unit of gain, has been determined for the three rations (Table 17). Figure 14 shows the decrease in feed efficiency as the chicks grew. The feed efficiency of the chicks receiving the liver meal ration was the best throughout the experiment. The feed efficiency of the chicks fed the APF ration was close to that of the liver meal ration at two weeks of age but decreased until it was equal to the basal ration at eight weeks and was the poorest thereafter.

Table 17. Feed Efficiency of Chicks Receiving the Basal, Liver Meal, and APF Rations

Ration	No. of chicks	Age in weeks	Total gain	Total feed consumed	Feed efficiency
			lb.	lb.	
Basal	30	2	3.96	8.75	2.21
	30	4	13.98	31.87	2.28
	30	6	27.48	70.5	2.57
	30	8	48.49	130.0	2.68
	30	10	72.88	208.5	2.86
	30	12	95.78	298.5	3.12
Liver Meal	30	2	5.58	11.0	1.97
	30	4	18.25	38.0	2.08
	30	6	33.54	82.0	2.44
	30	8	58.74	146.5	2.49
	30	10	81.99	224.0	2.73
	30	12	101.99	310.5	3.04
APF	30	2	5.13	10.5	2.05
	30	4	16.88	38.0	2.25
	30	6	32.28	82.5	2.56
	29	8	55.26	148.0	2.68
	29	10	78.07	230.0	2.95
	29	12	99.65	326.0	3.27

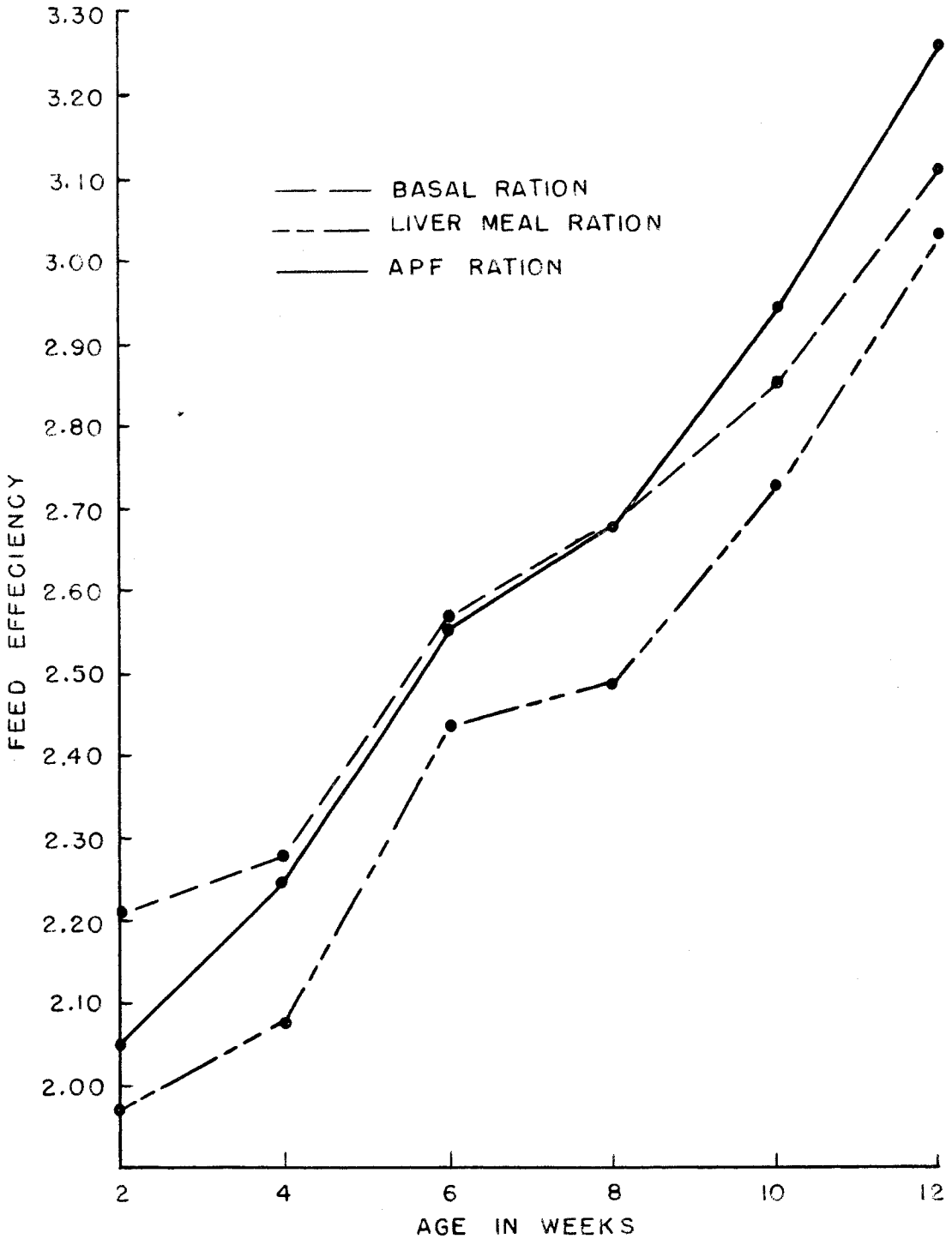


Figure 14. Feed efficiency of chicks receiving the basal, liver meal, and APF rations.

The following steps were taken in analyzing the feed efficiency data (Table 17) to determine whether or not there was a difference among rations:

	Basal	Liver Meal	APF	Total
	2.21	1.97	2.05	6.23
	2.28	2.08	2.25	6.61
	2.57	2.44	2.56	7.57
	2.68	2.49	2.68	7.85
	2.86	2.73	2.95	8.54
	3.12	3.04	3.27	9.43
Total	15.72	14.75	15.76	46.23
Means	2.62	2.46	2.63	

Correction term (C) = $\frac{46.23^2}{18}$ = 118.7341

Total S.S. = $2.21^2 + 2.28^2 + \ldots + 3.27^2 - C$ = 2.5016

Ration S.S.= $\frac{15.72^2 + 14.75^2 + 15.76^2}{6} - C$ = 0.1090

Period S.S.= $\frac{6.23^2 + 6.61^2 + \ldots + 9.43^2}{3} - C$ = 2.3622

Analysis of Variance

S.V.	D.F.	S.S.	M.S.
Rations	2	0.1090	0.0545**
Periods	5	2.3622	0.4724**
R x P (error)	10	0.0304	0.00304
Total	17	2.5016	

From the analysis of variance, there is a highly significant difference among rations and periods (weeks) with regard to feed efficiency. The differences among weeks are easily seen by gross inspection of the data. The effect of rations is not so apparent, but the F-value (17.93) obtained by dividing 0.0545 by 0.00304 is still larger than the F-value (7.56) at the 1% level with 2 and 10 d.f.

The L.S.D. of the means demonstrates that there is no appreciable difference in feed efficiency of the chicks receiving basal and APF rations, whereas there is a difference between the basal and liver meal rations and also between the APF and liver meal rations. These differences were significant at the 1% level. The calculations were made by multiplying the mean square of the error term (0.00304) by two, dividing the product by six, extracting the square root of the quotient, and then multiplying by the t-value at the 5 and 1% levels with 10 d.f. The L.S.D. at the 5% level is 0.07 and at the 1% level it is 0.10. Since the difference in the means between the basal and liver meal rations (0.16) and also between the APF and liver meal rations (0.17) is greater than 0.10, there is a highly significant difference in the feed efficiency of the chicks fed those rations.

C. Hemoglobin Values

Hemoglobin determinations were started when the chicks were four weeks of age and continued until the hemoglobin levels were determined for all the chicks. The hemoglobin levels of four chicks in each pen were then rechecked to see if there had been a change in the hemoglobin concentration with age. The last hemoglobin determinations were made

Table 18. Hemoglobin Values of Blood from Chicks Receiving the Basal Ration

	Chick no.	Sex	Age in days	Gm. hemoglobin per 100 ml. blood
Pen 1	101D	M	28	7.31
	101G	F	35	5.16
	101K	M	35	6.59
	104H	F	35	7.52
	103L	F	37	6.42
	105J	F	37	5.52
	108C	M	41	7.26
	109D	M	41	6.44
	106K	M	43	8.42
	108K	F	43	6.50
	101D	M	69	6.80
	101G	F	69	7.19
	101K	M	71	7.92
	104H	F	71	7.60
Pen 2	101J	F	45	6.58
	104I	M	45	6.63
	102G	M	48	6.00
	105C	M	48	7.00
	104L	F	50	6.78
	107H	M	50	8.16
	106E	M	52	6.87
	108H	M	52	6.73
	108L	F	55	7.07
	109K	F	55	6.32
	101J	F	73	8.04
	104I	M	73	7.08
	102G	M	76	6.99
	105C	M	76	6.76
Pen 3	102C	F	57	6.90
	102E	F	57	6.19
	104D	F	59	6.13
	104E	M	59	6.70
	106H	F	62	7.36
	108I	M	62	7.28
	106F	M	64	6.89
	109J	M	64	6.67
	109L	M	66	7.37
	109G	F	66	6.14
	102C	F	78	6.26
	102E	F	78	7.47
	104D	F	80	6.99
	104E	M	80	7.17

Table 19. Hemoglobin Values of Blood from Chicks Receiving the Liver Meal Ration

	Chick no.	Sex	Age in days	Gm. hemoglobin per 100 ml. blood
Pen 4	101F	F	28	6.99
	103I	F	35	6.71
	105I	F	35	6.05
	103G	F	35	7.21
	105H	F	37	5.91
	105E	M	37	7.01
	106J	M	41	6.83
	107L	M	41	6.37
	108F	M	43	6.67
	106G	M	43	6.28
	101F	F	69	7.24
	103I	F	69	6.45
	105I	F	71	7.28
	103G	F	71	7.69
Pen 5	102F	M	45	6.15
	102J	F	45	6.80
	102H	F	48	6.31
	104C	F	48	7.22
	104J	M	50	7.36
	106L	F	50	8.56
	107J	F	52	6.78
	107F	F	52	7.63
	107K	F	55	7.07
	108E	F	55	6.98
	102F	M	73	6.85
	102J	F	73	7.26
	102H	F	76	7.85
	104C	F	76	7.22
Pen 6	103D	F	57	6.68
	101E	F	57	7.03
	103H	M	59	6.48
	104F	M	59	6.22
	107C	F	62	8.29
	108D	M	62	6.53
	105F	M	64	7.15
	105K	F	64	7.67
	109H	M	66	7.10
	109E	F	66	6.31
	103D	F	78	6.80
	101E	F	78	6.39
	103H	M	80	7.26
	104F	M	80	6.85

Table 20. Hemoglobin Values of Blood from Chicks Receiving the APF Ration

	Chick no.	Sex	Age in days	Gm. hemoglobin per 100 ml. blood
Pen 7	101C	M	28	7.44
	102I	M	28	7.27
	102L	F	35	6.10
	103C	M	35	7.16
	106D	M	37	6.37
	105L	F	37	6.20
	102K	M	41	6.39
	104K	F	41	6.44
	104G	F	43	6.28
	109I	F	43	6.63
	101C	M	69	6.88
	102I	M	69	7.30
	102L	F	71	6.59
	103C	M	71	7.14
Pen 8	101I	M	45	6.59
	103F	M	45	6.98
	101H	M	48	6.04
	103E	M	48	6.48
	103J	F	50	6.02
	107D	F	50	6.74
	109C	M	52	6.24
	108J	F	52	6.64
	109F	F	55	6.81
	107G	M	55	6.18
	101I	M	73	7.03
	103F	M	73	7.22
	101H	M	76	7.49
	103E	M	76	6.89
Pen 9	102D	F	57	7.56
	101L	F	57	6.68
	106I	M	59	6.39
	105D	M	59	6.44
	106C	F	62	7.85
	107I	M	62	6.88
	103K	M	64	7.59
	107E	F	64	7.11
	105G	M	66	6.93
	108G	M	66	7.50
	102D	F	78	7.17
	101L	F	78	6.26
	106I	M	80	6.36
	105D	M	80	7.49

four days before the experiment was completed. Tables 18, 19, and 20 present the individual hemoglobin values of chicks receiving the basal, liver meal, and APF rations, respectively, while Table 21 shows the mean hemoglobin values of 90 chicks. The hemoglobin values of the four chicks in each pen used to check the effect of age on hemoglobin were not included in Table 21.

Table 21. The Mean and Standard Error of the Mean of Hemoglobin Values from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

	Gm. hemoglobin per 100 ml. blood		
Sex	Basal	Liver meal	APF
Male	7.02 ± 0.12	6.68 ± 0.14	6.76 ± 0.12
Female	6.47 ± 0.22	7.01 ± 0.19	6.70 ± 0.23

An analysis of variance of the hemoglobin values of male and female chicks is as follows:

Completed Analysis of Variance of Hemoglobin Values for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.9410	0.4705
Pens treated alike	6	1.3556	0.2256
Individuals	36	10.0721	0.2798
Total	44	12.3687	

Completed Analysis of Variance of Hemoglobin Values for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	2.3556	1.1778
Pens treated alike	6	4.0557	0.6760
Individuals	36	13.3663	0.3713
Total	44	19.7776	

These analyses reveal the absence of treatment and environmental effects in the hemoglobin values. The L.S.D. between the means of male chicks receiving basal and liver meal rations is 0.41, basal and APF rations 0.37, and liver meal and APF rations 0.40. The L.S.D. between the means of female chicks receiving basal and liver meal rations is 0.44, basal and APF rations 0.48, and liver meal and APF rations 0.45. By comparing the differences between the means in Table 21 with the L.S.D. for the various rations, the hemoglobin mean for female chicks fed the basal ration is observed as being significantly different from those fed the liver meal ration. This difference was not observed in the above analysis of variance for female chicks which indicates that the L.S.D. should be determined when more than two treatments are involved. The difference (0.54) between the means of hemoglobin values of female chicks fed the basal and liver meal rations (Table 21) was less than the 1% level of significance (0.59).

The mean and standard error of the mean for the hemoglobin values of chicks for age 1 is 6.66 $\pm$ 0.06 and for

age 2, 7.09 ± 0.06. These means are obtained from the hemoglobin values (Tables 18, 19, and 20) of the first four chicks in each pen (age 1) and from the second hemoglobin values (age 2) of the same birds determined later. An analysis of variance of these data is as follows:

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	3.3627	3.3627**
Treatments (T)	2	0.0001	0.00005
A x T	2	0.4988	0.2494
Experimental error	12	1.5652	0.1304
Sampling error	54	14.3864	0.2664
Total	71	19.8132	

The hemoglobin values for the two ages of chicks have come from different populations. By dividing the mean square for ages, 3.3627, by the mean square for experimental error, 0.1304, the quotient (25.79) is found to be larger than the $F_{.01}$ value (9.33) for 1 and 12 d.f. The difference between the hemoglobin means for the two ages is 0.43. The L.S.D. was found to be 0.19 and 0.26 for the 5 and 1% levels of significance, respectively.

D. Erythrocyte Counts

Erythrocyte counts were made on the same blood samples from which hemoglobin values were determined. Tables 22, 23, and 24 present the individual counts and Table 25 shows the mean and standard deviation of the mean for 90 male and

Table 22. Erythrocyte Counts of Blood from Chicks Receiving the Basal Ration

	Chick no.	Sex	Age in days	Erythrocytes per cu. mm. blood 10^6	Acidophilic granulocytes*
	101D	M	28	2.245	18.5
	101G	F	35	2.12	13.0
	101K	M	35	2.405	49.5
	104H	F	35	3.10	40.0
	103L	F	37	2.96	18.0
Pen 1	105J	F	37	2.275	37.0
	108C	M	41	2.57	19.0
	109D	M	41	2.27	33.0
	106K	M	43	3.07	35.5
	108K	F	43	2.85	30.4
	101D	M	69	2.64	43.0
	101G	F	69	2.67	34.0
	101K	M	71	2.04	37.0
	104H	F	71	3.125	48.0
	101J	F	45	2.64	37.5
	104I	M	45	2.87	46.0
	102G	M	48	2.26	42.5
	105C	M	48	2.09	35.5
	104L	F	50	2.48	37.5
Pen 2	107H	M	50	3.39	41.5
	106E	M	52	2.455	24.0
	108H	M	52	2.395	34.5
	108L	F	55	2.505	30.0
	109K	F	55	2.76	37.0
	101J	F	73	3.155	29.0
	104I	M	73	2.65	60.5
	102G	M	76	2.40	29.0
	105C	M	76	2.385	34.5
	102C	F	57	2.46	23.0
	102E	F	57	2.54	39.0
	104D	F	59	2.365	20.0
	104E	M	59	2.79	40.0
	106H	F	62	2.44	39.0
Pen 3	108I	M	62	2.405	39.5
	106F	M	64	2.38	31.0
	109J	M	64	2.375	23.5
	109L	M	66	2.345	29.5
	109G	F	66	2.675	39.5
	102C	F	78	2.30	11.5
	102E	F	78	3.205	46.0
	104D	F	80	2.595	35.0
	104E	M	80	2.535	39.0

*Acidophilic granulocytes counted over entire ruled area of hemacytometer to be used in calculating total leucocyte count

Table 23. Erythrocyte Counts of Blood from Chicks Receiving the Liver Meal Ration

	Chick no.	Sex	Age in days	Erythrocytes per cu. mm. blood 10^6	Acidophilic granulocytes*
	101F	F	28	2.40	21.75
	103I	F	35	2.64	23.0
	105I	F	35	2.575	45.0
	103G	F	35	2.39	21.0
	105H	F	37	2.49	35.5
Pen 4	105E	M	37	2.61	38.5
	106J	M	41	2.69	31.5
	107L	M	41	2.57	24.0
	108F	M	43	3.01	48.5
	106G	M	43	2.845	31.0
	101F	F	69	2.585	35.5
	103I	F	69	2.37	20.5
	105I	F	71	2.795	45.0
	103G	F	71	2.485	19.0
	102F	M	45	2.615	71.0
	102J	F	45	2.68	33.0
	102H	F	48	2.40	30.0
	104C	F	48	2.515	33.5
	104J	M	50	2.86	39.0
Pen 5	106L	F	50	2.42	33.0
	107J	F-	52	2.945	67.0
	107F	F	52	2.72	32.5
	107K	F	55	2.66	25.5
	108E	F	55	2.905	25.5
	102F	M	73	2.34	40.5
	102J	F	73	2.35	44.0
	102H	F	76	2.93	54.5
	104C	F	76	2.90	22.0
	103D	F	57	2.62	26.0
	101E	F	57	2.465	31.5
	103H	M	59	2.42	20.5
	104F	M	59	2.475	20.0
	107C	F	62	3.385	36.0
Pen 6	108D	M	62	2.25	21.0
	105F	M	64	2.57	39.0
	105K	F	64	2.89	26.5
	109H	M	66	2.905	83.0
	109E	F	66	2.53	35.0
	103D	F	78	1.88	30.5
	101E	F	78	2.19	62.5
	103H	M	80	2.115	24.0
	104F	M	80	2.325	23.0

*Acidophilic granulocytes counted over entire ruled area of hemacytometer to be used in calculating total leucocyte count

Table 24. Erythrocyte Counts of Blood from Chicks Receiving the APF Ration

	Chick no.	Sex	Age in days	Erythrocytes per cu. mm. blood 10^6	Acidophilic granulocytes*
	101C	M	28	2.935	18.0
	102I	M	28	2.795	124.5
	102L	F	35	2.44	42.5
	103C	M	35	2.685	30.0
	106D	M	37	2.02	28.0
Pen 7	105L	F	37	2.18	16.0
	102K	M	41	2.54	8.0
	104K	F	41	2.31	24.5
	104G	F	43	2.31	32.0
	109I	F	43	2.51	43.5
	101C	M	69	2.48	26.0
	102I	M	69	2.885	27.0
	102L	F	71	2.37	37.5
	103C	M	71	2.435	28.5
	101I	M	45	2.63	44.5
	103F	M	45	2.65	51.5
	101H	M	48	2.305	23.0
	103E	M	48	2.72	25.5
	103J	F	50	2.50	27.5
Pen 8	107D	F	50	2.73	25.0
	109C	M	52	2.73	28.0
	108J	F	52	2.725	34.5
	109J	F	55	2.55	23.0
	107G	M	55	2.42	20.0
	101I	M	73	3.04	44.5
	103F	M	73	2.515	24.5
	101H	M	76	2.47	29.0
	103E	M	76	2.525	13.0
	102D	F	57	2.87	19.5
	101L	F	57	2.70	17.0
	106I	M	59	2.495	16.5
	105D	M	59	2.25	30.5
	106C	F	62	2.765	25.5
Pen 9	107I	M	62	2.365	27.0
	103K	M	64	2.845	11.0
	107E	F	64	2.435	22.5
	105G	M	66	2.79	19.0
	108G	M	66	2.845	27.0
	102D	F	78	2.11	27.5
	101L	F	78	2.765	23.0
	106I	M	80	2.365	14.5
	105D	M	80	2.585	39.0

*Acidophilic granulocytes counted over entire ruled area of hemacytometer to be used in calculating total leucocyte count

female chicks receiving the three rations. The erythrocyte counts (Tables 22, 23, and 24) of the four chicks in each pen used to check the effect of age were not included in Table 25.

Table 25. The Mean and Standard Error of the Mean of Erythrocyte Counts from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

Sex	Erythrocytes per cu. mm. blood 10^6		
	Basal	Liver Meal	APF
Male	2.519688±0.043269	2.651667±0.049962	2.589412±0.041977
Female	2.583636±0.082240	2.646111±0.072529	2.540385±0.085344

The analyses of variance of the erythrocyte counts from male and female chicks receiving the three rations are as follows:

Completed Analysis of Variance for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.121274	0.060637
Pens treated alike	6	0.179731	0.029955
Individuals	36	3.117803	0.086606
Total	44	3.418808	

Completed Analysis of Variance for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.087572	0.043786
Pens treated alike	6	0.568122	0.094687
Individuals	36	1.981050	0.055029
Total	44	2.636744	

The treatments and environmental conditions produced no changes in the erythrocyte counts. These counts can be considered as coming from the same population. The differences between the means (Table 25) were less than the L.S.D.'s in all cases.

An analysis of variance determining if age caused a significant change in the erythrocyte counts of chicks is as follows:

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	0.000028	0.000028
Treatments (T)	2	0.156368	0.078184
A x T	2	0.209306	0.104653
Experimental error	12	0.732079	0.061007
Sampling error	54	4.190419	0.077600
Total	71	5.288200	

There is no significant difference in the erythrocyte counts between the two ages of chicks. The mean and standard error of the mean of the erythrocyte counts of chicks per cu. mm. of blood (age 1) were 2,543,194 $\pm$ 41,167 and (age 2) 2,541,944 $\pm$ 41,167.

E. Total and Differential Leucocyte Counts

The total and differential leucocyte counts are presented in Tables 26, 27, and 28. The mean and standard error of the mean of these counts are shown in Table 29. The counts from the four chicks in each pen used to check the significance of age are not included in Table 29.

Table 26. Total and Differential Leucocyte Counts per cu. mm. of Blood from Chicks Receiving the Basal Ration

	Chick no.	Sex	Age in days	Eosino-phils	Baso-phils	Lympho-cytes	Hetero-phils	Mono-cytes	Total leuco-cytes
	101D	M	28	164.4	246.7	10,688.6	3,946.6	1,397.7	16,444
	101G	F	35	222.2	111.1	3,814.6	2,666.5	592.6	7,407
	101K	M	35	492.5	164.2	19,701.0	10,507.2	1,970.1	32,835
	104H	F	35	459.8	306.5	19,003.6	8,429.0	2,452.1	30,651
	103L	F	37	117.7	235.3	17,646.8	3,882.3	1,647.0	23,529
Pen	105J	F	37		171.3	22,782.2	8,222.2	3,083.3	34,259
1	108C	M	41	272.4		8,716.8	3,949.8	681.0	13,620
	109D	M	41	99.1		10,504.6	7,234.3	1,982.0	19,820
	106K	M	43	369.8	123.3	15,531.4	7,519.2	1,109.4	24,653
	108K	F	43	193.7		9,973.0	6,584.1	2,614.3	19,365
	101D	M	69	955.6	159.3	20,226.0	8,600.0	1,911.1	31,852
	101G	F	69	503.7	125.9	16,118.4	6,296.3	2,140.7	25,185
	101K	M	71	761.3		21,012.6	7,461.0	1,218.1	30,453
	104H	F	71	561.4	748.5	23,579.0	10,105.3	2,432.8	37,427
	101J	F	45	406.5	609.8	29,674.5	7,926.8	2,032.5	40,650
	104I	M	45	157.3		19,658.1	10,065.0	1,572.7	31,453
	102G	M	48	566.7	188.9	24,366.8	8,877.8	3,777.8	37,778
	105C	M	48	213.2	319.8	11,833.2	7,675.6	1,279.3	21,321
	104L	F	50		277.8	17,639.0	8,333.4	1,527.8	27,778
Pen	107H	M	50		146.4	19,030.1	9,222.3	878.3	29,277
2	106E	M	52	217.7	108.9	13,387.9	5,115.7	2,938.8	21,769
	108H	M	52			26,922.6	7,666.7	1,069.8	35,659
	108L	F	55	158.7	317.5	22,380.9	6,507.9	2,381.0	31,746
	109K	F	55	274.1	137.0	17,814.6	7,948.0	1,233.3	27,407
	101J	F	73	345.2		14,385.0	6,099.2	2,186.5	23,016
	104I	M	73	689.5	172.4	17,581.2	12,755.0	3,274.9	34,473
	102G	M	76	169.6	169.6	24,590.6	6,274.8	2,713.4	33,918
	105C	M	76	1,052.3		20,594.5	6,614.3	1,803.9	30,065
	102C	F	57	494.6	329.8	25,720.5	4,616.5	1,813.6	32,975
	102E	F	57	240.7	722.2	37,314.7	8,425.9	1,444.4	48,148
	104D	F	59	888.9	222.2	38,221.8	3,555.5	1,555.5	44,444
	104E	M	59	150.7		20,339.1	8,738.3	904.0	30,132
Pen	106H	F	62	228.1		34,438.6	8,438.6	2,508.8	45,614
3	108I	M	62	702.2		24,226.6	8,075.5	2,106.7	35,111
	106F	M	64	260.0	130.0	17,677.3	6,629.0	1,299.8	25,996
	109J	M	64	284.9		12,153.6	4,937.4	1,614.2	18,990
	109L	M	66	204.9	102.4	13,008.6	6,350.7	819.4	20,486
	109G	F	66	411.5		17,007.2	8,366.5	1,645.9	27,431
	102C	F	78	67.3		10,020.3	2,488.3	874.3	13,450
	102E	F	78	179.3		23,134.2	10,042.8	2,510.7	35,867
	104D	F	80	527.3		16,873.6	7,250.4	1,713.7	26,365
	104E	M	80		216.7	31,849.8	8,666.6	2,600.0	43,333

Table 27. Total and Differential Leucocyte Counts per cu. mm. of Blood from Chicks Receiving the Liver Meal Ration

	Chick no.	Sex	Age in days	Eosino-phils	Baso-phils	Lympho-cytes	Hetero-phils	Mono-cytes	Total leuco-cytes
	101F	F	28	91.2		11,946.6	4,742.1	1,459.1	18,239
	103I	F	35	309.8	154.9	23,386.9	4,801.3	2,323.2	30,976
	105I	F	35	158.7		20,476.2	9,841.3	1,269.8	31,746
	103G	F	35	119.7	179.5	6,701.0	4,547.1	418.8	11,966
	105H	F	37	464.1	928.1	19,645.0	7,424.9	2,475.0	30,937
Pen	105E	M	37	192.3		9,132.4	8,363.3	1,538.1	19,226
4	106J	M	41	444.4		13,222.1	6,555.5	2,000.0	22,222
	107L	M	41	213.3	142.2	8,319.9	5,119.9	426.7	14,222
	108F	M	43	295.3		16,092.8	10,482.4	2,657.5	29,528
	106G	M	43	336.0	168.0	25,035.0	6,552.8	1,512.2	33,604
	101F	F	69	154.7		19,490.3	7,734.3	3,557.8	30,937
	103I	F	69	569.5		17,197.4	3,986.2	1,025.0	22,778
	105I	F	71	634.9	317.5	19,365.1	9,365.1	2,063.5	31,746
	103G	F	71	333.3	222.2	15,999.8	3,888.9	1,777.8	22,222
	102F	M	45	190.1		18,629.3	15,587.8	3,611.8	38,019
	102J	F	45	222.2		12,888.8	7,111.0	2,000.0	22,222
	102H	F	48	505.1	101.0	12,222.2	6,161.6	1,212.1	20,202
	104C	F	48	132.9		15,952.2	7,311.4	3,190.4	26,587
	104J	M	50	122.1	244.1	14,281.6	8,544.6	1,220.7	24,413
Pen	106L	F	50	396.4	198.2	30,324.6	6,937.0	1,783.8	39,640
5	107J	F	52		402.4	23,540.4	14,888.8	1,408.4	40,240
	107F	F	52	136.3		18,260.2	7,086.0	1,771.5	27,254
	107K	F	55	202.4		14,065.4	5,464.3	506.0	20,238
	108E	F	55	202.4	202.4	32,583.2	5,464.3	2,023.8	40,476
	102F	M	73	147.5		19,475.3	8,852.4	1,032.8	29,508
	102J	F	73	564.1	188.0	24,820.6	9,213.7	2,820.5	37,607
	102H	F	76	698.7		30,044.8	11,412.4	4,425.2	46,581
	104C	F	76		174.6	27,936.8	4,888.9	1,920.7	34,921
	103D	F	57	608.2		52,304.3	5,169.6	2,736.9	60,819
	101E	F	57	325.6		22,139.4	6,674.4	3,418.6	32,558
	103H	M	59	93.0	93.0	12,922.8	4,462.6	1,022.7	18,594
	104F	M	59	238.1	238.1	10,079.4	4,206.4	1,111.1	15,873
	107C	F	62	444.4	222.2	33,555.2	7,555.5	2,666.6	44,444
Pen	108D	M	62			25,747.2	4,666.7	1,770.1	32,184
6	105F	M	64	517.4	129.4	16,040.0	8,149.4	1,034.8	25,871
	105K	F	64	190.0		11,397.6	5,698.8	1,709.6	18,996
	109H	M	66	302.4		36,889.1	18,142.2	5,140.3	60,474
	109E	F	66	691.4		25,061.8	7,086.4	1,728.4	34,568
	103D	F	78			18,215.5	6,777.8	3,247.7	28,241
	101E	F	78	964.5		16,203.6	12,924.3	8,487.6	38,580
	103H	M	80	152.4	76.2	8,838.0	5,180.9	990.5	15,238
	104F	M	80	237.7		16,641.1	4,873.5	2,020.7	23,773

Table 28. Total and Differential Leucocyte Counts per cu. mm. of Blood from Chicks Receiving the APF Ration

	Chick no.	Sex	Age in days	Eosino-phils	Baso-phils	Lympho-cytes	Hetero-phils	Mono-cytes	Total leuco-cytes
	101C	M	28		222.2	37,333.0	4,000.0	2,888.9	44,444
	102I	M	28	189.5	379.0	8,338.0	27,477.5	1,516.0	37,900
	102L	F	35	349.8	524.7	23,261.0	9,094.5	1,749.0	34,979
	103C	M	35	115.0	229.9	13,103.7	6,551.9	2,988.6	22,989
	106D	M	37	455.3	607.0	21,549.9	5,766.9	1,972.9	30,352
Pen	105L	F	37	52.3	52.3	5,908.8	3,503.4	941.2	10,458
7	102K	M	41	114.7	172.1	8,545.2	1,663.2	975.0	11,470
	104K	F	41	93.9	187.7	11,452.1	5,350.6	1,689.7	18,774
	104G	F	43	245.2	245.2	7,846.6	6,865.7	1,144.3	16,347
	109I	F	43	767.2	153.4	18,412.8	8,899.5	2,455.0	30,688
	101C	M	69	346.7	115.6	16,524.4	5,431.1	693.3	23,111
	102I	M	69	264.7	88.2	11,029.4	5,735.3	529.4	17,647
	102L	F	71	520.8		40,364.3	7,812.5	3,385.4	52,083
	103C	M	71	372.6	186.3	27,941.3	5,960.8	2,794.1	37,255
	101I	M	45	423.8	141.3	14,833.4	9,465.1	3,390.5	28,254
	103F	M	45	168.3	168.3	19,859.4	11,276.1	2,187.9	33,660
	101H	M	48			18,655.9	5,111.2	1,788.9	25,556
	103E	M	48	213.8		14,861.9	5,452.9	855.4	21,384
	103J	F	50		370.4	28,148.1	6,111.1	2,407.4	37,037
Pen	107D	F	50	370.4		30,000.0	5,185.2	1,481.5	37,037
8	109C	M	52	129.6		18,277.8	6,092.6	1,425.9	25,926
	108J	F	52	196.6		29,880.2	7,470.0	1,769.2	39,316
	109F	F	55	94.7	378.6	12,872.4	5,016.5	567.9	18,930
	107G	M	55			19,259.0	4,444.4	987.6	24,691
	101I	M	73	534.5		14,165.3	9,354.5	2,672.7	26,727
	103F	M	73	272.2	136.1	20,144.3	5,172.2	1,497.2	27,222
	101H	M	76	161.1		23,199.8	6,283.3	2,577.8	32,222
	103E	M	76	78.1		12,492.8	2,810.9	234.2	15,616
	102D	F	57	201.6		14,713.2	4,131.8	1,108.5	20,155
	101L	F	57		107.9	15,974.4	3,777.7	1,727.0	21,587
	106I	M	59	261.9	261.9	20,952.0	3,404.7	1,309.5	26,190
	105D	M	59			29,934.9	6,777.7	941.4	37,654
	106C	F	62	145.3	145.3	21,649.7	5,521.4	1,598.3	29,060
Pen	107I	M	62	125.0		17,000.0	5,875.0	2,000.0	25,000
9	103K	M	64	325.9		28,681.8	2,118.6	1,466.7	32,593
	107E	F	64	263.2		17,763.3	4,736.9	3,552.7	26,316
	105G	M	66	392.8		14,630.3	3,829.4	785.5	19,638
	108G	M	66	510.6		17,234.1	5,489.4	2,297.9	25,532
	102D	F	78	218.3	436.5	35,793.8	5,892.9	1,309.5	43,651
	101L	F	78	269.0	269.0	41,426.8	4,842.1	6,994.1	53,801
	106I	M	80	420.3	280.2	22,835.5	2,801.9	1,681.1	28,019
	105D	M	80	428.0		11,448.5	8,238.6	1,283.9	21,399

Table 29. The Mean and Standard Error of the Mean of Total Leucocyte and Differential Counts per cu. mm. of Blood from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

	Sex	Basal	Liver meal	APF
Total leucocytes	M	25,959.0±1,893.3	27,852.5±2,186.1	27,837.2±1,836.7
" "	F	31,528.9±4,155.8	30,672.7±3,665.0	26,206.5±4,312.6
Lymphocytes	M	16,734.1±1,620.5	17,199.3±1,871.2	19,003.0±1,572.1
"	F	22,388.0±4,036.7	21,469.5±3,560.1	18,298.7±4,189.1
Heterophils	M	7,281.9± 997.4	8,402.8±1,151.7	6,752.7± 967.6
"	F	6,707.4± 533.5	6,887.0± 470.5	5,820.3± 553.6
Monocytes	M	1,587.6± 177.7	1,920.5± 205.2	1,751.7± 172.4
"	F	1,895.2± 187.9	1,894.6± 165.7	1,707.1± 195.0
Eosinophils	M	259.7± 35.5	245.4± 41.0	201.5± 34.5
"	F	292.6± 72.9	288.9± 64.3	213.8± 75.7
Basophils	M	95.7± 55.0	84.6± 63.5	128.3± 53.4
"	F	245.8± 54.1	132.7± 47.7	166.6± 56.1

The analyses of variance of the various counts per cu. mm. of blood from male and female chicks receiving the three rations are as follows:

Completed Analysis of Variance of Total Leucocyte Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	36,621,907	18,310,953.5
Pens treated alike	6	344,100,938	57,350,156.3
Individuals	36	3,209,683,830	89,157,884.1
Total	44	3,590,406,675	

Completed Analysis of Variance of Total Leucocyte Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	222,400,078	111,200,039
Pens treated alike	6	1,450,709,223	241,784,870*
Individuals	36	3,494,673,712	97,074,270
Total	44	5,167,783,013	

Completed Analysis of Variance of Lymphocyte Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	46,785,011.5	23,392,505.8
Pens treated alike	6	252,099,820.1	42,016,636.6
Individuals	36	1,857,376,513.8	51,593,792.0
Total	44	2,156,261,345.4	

Completed Analysis of Variance of Lymphocyte Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	124,640,865	62,320,432.5
Pens treated alike	6	1,368,787,383	228,131,230.0*
Individuals	36	2,660,664,303	73,907,341.0
Total	44	4,154,092,551	

Completed Analysis of Variance of Heterophil Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	19,395,889.0	9,697,944.5
Pens treated alike	6	95,504,934.2	15,917,489.0
Individuals	36	726,948,777.5	20,193,021.5
Total	44	841,849,600.7	

Completed Analysis of Variance of Heterophil Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	9,279,227.0	4,639,613.5
Pens treated alike	6	23,908,057.8	3,984,676.3
Individuals	36	170,631,730.4	4,739,770.3
Total	44	203,819,015.2	

Completed Analysis of Variance of Monocyte Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	764,963.0	382,481.5
Pens treated alike	6	3,031.662.5	505,277.1
Individuals	36	36,464,477.9	1,012,902.2
Total	44	40,261,103.4	

Completed Analysis of Variance of Monocyte Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	325,934.4	162,967.2
Pens treated alike	6	2,966,231.0	494,371.8
Individuals	36	21,040,341.4	584,453.9
Total	44	24,332,506.8	

Completed Analysis of Variance of Eosinophil Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	30,049.3	15,024.7
Pens treated alike	6	121,289.7	20,215.0
Individuals	36	1,123,107.0	31,197.4
Total	44	1,274,446.0	

Completed Analysis of Variance of Eosinophil Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	54,470.5	27,235.3
Pens treated alike	6	446,748.0	74,458.0
Individuals	36	1,374,087.7	38,169.1
Total	44	1,875,306.2	

Completed Analysis of Variance of Basophil Counts for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	15,663.4	7,831.7
Pens treated alike	6	290,333.3	48,388.9**
Individuals	36	450,062.2	12,501.7
Total	44	756,058.9	

Completed Analysis of Variance of Basophil Counts for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	102,870.2	51,435.1
Pens treated alike	6	245,383.8	40,897.3
Individuals	36	1,600,213.6	44,450.4
Total	44	1,948,467.6	

From the above analyses, there is no evidence of treatment effects, whereas environmental effects were present in the total leucocyte and lymphocyte counts of females at the 5% level of significanace and also in the basophil counts of males at the 1% level. The differences between the treatment means were less than the L.S.D.'s. Therefore, no significant difference exists among the three treatment means in each row of Table 29.

The effect of age on the total and differential leucocyte counts (Tables 26, 27, and 28) has been summarized in Table 30. The difference in age of the two groups designated as age 1 and age 2 varies from 21 to 37 days.

Table 30. The Mean and Standard Error of the Mean of Total and Differential Leucocyte Counts per cu. mm. of Blood from Chicks of Two Different Ages

	Age 1	Age 2
Total leucocytes	29,361.1 ± 1,857.5	30,730.3 ± 1,857.5
Lymphocytes	19,772.4 ± 1,733.4	20,876.6 ± 1,733.4
Heterophils	7,293.6 ± 623.5	7,002.5 ± 623.5
Monocytes	1,861.6 ± 186.7	2,344.5 ± 186.7
Eosinophils	260.4 ± 39.5	393.2 ± 39.5
Basophils	167.3 ± 31.4	113.4 ± 31.4

The following analyses of variance were made in order to determine the effect of age on the total and differential leucocyte counts:

Analysis of Variance for Total Leucocyte Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	33,740,374.22	33,740,374.22
Treatments (T)	2	61,537,641.03	30,768,820.52
A x T	2	42,778,771.53	21,389,385.76
Experimental error	12	1,490,467,383.50	124,205,610.00
Sampling error	54	5,828,769,529.00	107,940,176.46
Total	71	7,457,293,699.28	

Analysis of Variance for Lymphocyte Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	21,948,857.02	21,948,857.02
Treatments (T)	2	73,518,379.26	36,759,189.63
A x T	2	90,631,699.21	45,315,849.60
Experimental error	12	1,298,062,886.28	108,171,907.19
Sampling error	54	4,148,334,446.42	76,821,008.27
Total	71	5,632,496,268.19	

Analysis of Variance for Heterophil Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	1,525,465.89	1,525,465.89
Treatments (T)	2	2,837,175.83	1,418,587.92
A x T	2	32,215,370.62	16,107,685.31
Experimental error	12	167,930,700.14	13,994,225.01
Sampling error	54	722,192,910.77	13,373,942.79
Total	71	926,701,623.25	

Analysis of Variance for Monocyte Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	4,197,559.97	4,197,559.97
Treatments (T)	2	2,858,791.58	1,429,395.79
A x T	2	942,672.53	471,336.26
Experimental error	12	15,049,649.08	1,254,137.42
Sampling error	54	101,769,266.49	1,884,616.05
Total	71	124,817,939.65	

Analysis of Variance for Eosinophil Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	317,395.99	317,395.99*
Treatments (T)	2	424,029.16	212,014.58
A x T	2	8,758.85	4,379.43
Experimental error	12	675,490.92	56,290.91
Sampling error	54	2,943,047.41	54,500.88
Total	71	4,368,722.33	

Analysis of Variance for Basophil Counts

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	52,271.68	52,271.68
Treatments (T)	2	198,153.73	99,076.86
A x T	2	71,547.22	35,773.61
Experimental error	12	426,326.46	35,727.21
Sampling error	54	1,342,892.63	24,868.38
Total	71	2,091,191.72	

The eosinophil counts changed significantly with age. The difference in the eosinophil means (Table 30) is 132.8, whereas the L.S.D. is 121.9 at the 5% level. The difference was not significant at the 1% level.

F. Sedimentation Rates

The sedimentation rates of erythrocytes of the blood samples were determined at one-half, one, two, three, and six hour intervals after the hematocrit tubes were filled. Figure 15 demonstrates the sedimentation rack and tubes used to determine the sedimentation rates. Tables 31, 32, and 33 show the sedimentation rates of the erythrocytes from chicks receiving the three rations. Table 34 presents the mean and standard error of the mean of the sedimentation rates of 90 chicks receiving the three rations. The sedimentation rates (Tables 31, 32, and 33) of the four chicks in each pen used to check the effect of age were not included in Table 34.

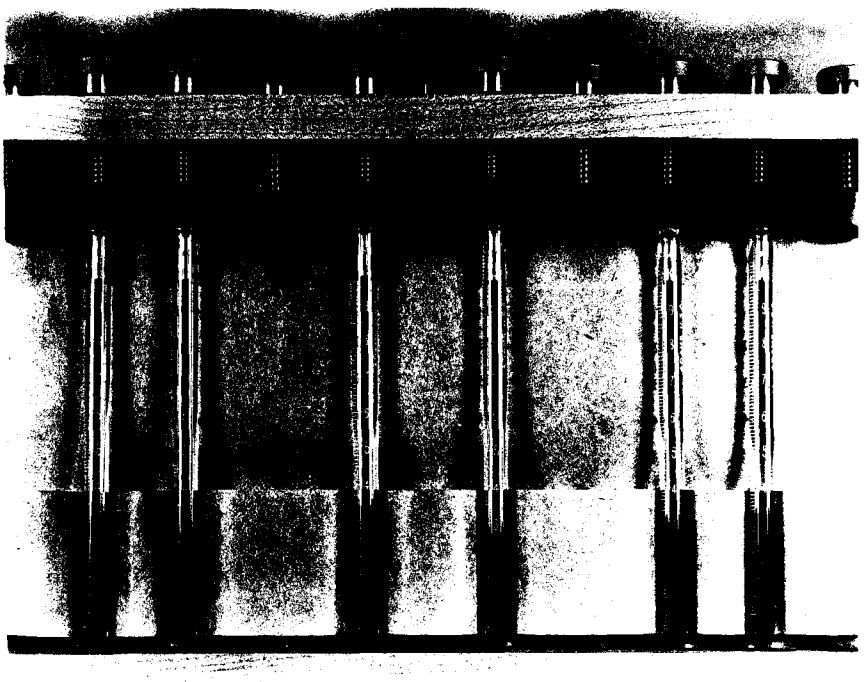


Figure 15. Sedimentation rack and tubes used in determining the sedimentation rate of erythrocytes.

Table 31. Sedimentation Rates of Erythrocytes Expressed in mm. from Chicks Receiving the Basal Ration

	Chick no.	Sex	Age in days	0.5 hr.	1 hr.	2 hr.	3 hr.	6 hr.
	101P	M	28	2	4	8	9	11
	101G	F	35	2	3	5	12	24
	101K	M	35	2	3	11	27	40
	104H	F	35	0.5	1	3	11	25
	103L	F	37	0.5	1	2	4	14
Pen 1	105J	F	37				0.5	2
	108C	M	41	2	6	7	8	21
	109D	M	41	0.5	2	4	7	15
	106K	M	43		1	2	5	10
	108K	F	43	0.5	2	3	5	11
	101D	M	69	0.5	1.5	3	6	14
	101G	F	69	1	2	5	9	19
	101K	M	71	0.5	1.5	4	7	14
	104H	F	71		1	3	5	12
	101J	F	45			0.5	2	11
	104I	M	45	0.5	2	4	6	13
	102G	M	48	1	3	5	8	16
	105C	M	48	1	2	4	7	15
	104L	F	50	1	2	5	8	16
Pen 2	107H	M	50	0.5	1	3	5	11
	106E	M	52	0.5	1	3	5	13
	108H	M	52	1.0	2	4	7	16
	108L	F	55		1	3	5	12
	109K	F	55	0.5	1	4	6	13
	101J	F	73	0.5	1	3	5	12
	104I	M	73	0.5	1	3	6	14
	102G	M	76	0.5	1.5	3.5	5	13
	105C	M	76	1	2	4	6	15
	102C	F	57		1	4	6	14
	102E	F	57	1	2	6	8	17
	104D	F	59	0.5	1.5	8	15	28
	104E	M	59		1	3	5	10
	106H	F	62		1	4	7	14
Pen 3	108I	M	62	0.5	1.5	4	8	15
	106F	M	64	0.5	1	3	6	15
	109J	M	64	0.5	2	6	17	32
	109L	M	66	0.5	1.5	5	8	16
	109G	F	66	0.5	1	4	7	15
	102C	F	78			0.5	1	3
	102E	F	78	0.5	2	4	6	14
	104D	F	80	0.5	4	8	11.5	20
	104E	M	80	1	2.5	4	6	12

Table 32. Sedimentation Rates of Erythrocytes Expressed in mm. from Chicks Receiving the Liver Meal Ration

	Chick no.	Sex	Age in days	0.5 hr.	1 hr.	2 hr.	3 hr.	6 hr.
	101F	F	28	0.5	1	3	5	14
	103I	F	35	0.5	1	3	10	22
	105I	F	35	1	2	3	8	18
	103G	F	35	0.5	1	3	7	20
	105H	F	37	1	5	7	13	20
Pen 4	105E	M	37	1	2	3	5	13
	106J	M	41	0.5	2	3	5	12
	107L	M	41	0.5	1	2	5	14
	108F	M	43		1	2	4	10
	106G	M	43	0.5	2	4	6	12
	101F	F	69	1	1.5	3	7	13
	103I	F	69	1	2.5	5	9	17
	105I	F	71		1	3	5	12
	103G	F	71		0.5	3	6	16
	102F	M	45	0.5	1	3	6	16
	102J	F	45	0.5	1	3	5	12
	102H	F	48	0.5	2	3	6	11
	104C	F	48	1.0	2	3	6	12
	104J	M	50	0.5	1	3	6	16
Pen 5	106L	F	50			1	2	5
	107J	F	52	0.5	1	3	5	12
	107F	F	52	0.5	1	3	5	12
	107K	F	55	0.5	1	4	6	12
	108E	F	55		1	3	5	11
	102F	M	73	0.5	1	3.5	6	15
	102J	F	73	1	1.5	4	7	16
	102H	F	76		1	3	5	12
	104C	F	76	0.5	2	4	6	18
	103D	F	57		1.5	3	5	13
	101E	F	57	0.5	1.5	5	7	15
	103H	M	59					
	104F	M	59	0.5	1.5	4	7	13
	107C	F	62		1	3	5	14
Pen 6	108D	M	62	0.5	2	5	8	14
	105F	M	64	0.5	1.5	3	5	14
	105K	F	64		0.5	2	4	10
	109H	M	66	0.5	1	5	9	16
	109E	F	66	0.5	1	4	7	15
	103D	F	78	1	3	5	7	16
	101E	F	78	0.5	2	4	6.5	15
	103H	M	80					
	104F	M	80	1	3	5	7	13

Table 33. Sedimentation Rates of Erythrocytes Expressed in mm. from Chicks Receiving the APF Ration

	Chick no.	Sex	Age in days	0.5 hr.	1 hr.	2 hr.	3 hr.	6 hr.
	101C	M	28	0.5	1	3	5	11
	102I	M	28	1	2	6	9	18
	102L	F	35	1	2	4	9	21
	103C	M	35	1	2	3	7	19
	106D	M	37	4	9	16	24	40
Pen 7	105L	F	37				1	5
	102K	M	41	0.5	2	3	5	13
	104K	F	41					
	104G	F	43	0.5	2	3	6	13
	109I	F	43		2	4	6	13
	101C	M	69	0.5	1	3	7	13
	102I	M	69	1	1.5	3	6	13
	102L	F	71	0.5	1	4	6	14
	103C	M	71	0.5	1	3.5	6	13
	101I	M	45					0.5
	103F	M	45	0.5	1	3	5	13
	101H	M	48	1	3	5	8	16
	103E	M	48	0.5	2	4	8	15
	103J	F	50	1	2	5	8	17
Pen 8	107D	F	50	1	2	5	8	17
	109C	M	52	0.5	1	4	7	17
	108J	F	52	0.5	1	4	6	14
	109F	F	55		1	4	7	15
	107G	M	55	0.5	1	4	7	15
	101I	M	73	0.5	1	3	5	11
	103F	M	73	0.5	1	3	5	13
	101H	M	76	0.5	1.5	3	5	11
	103E	M	76					
	102D	F	57	0.5	1	3	5	13
	101L	F	57	0.5	1.5	4	6	13
	106I	M	59		1.5	4	7	14
	105D	M	59	0.5	2	5	10	20
	106C	F	62		1.5	4	7	12
Pen 9	107I	M	62		0.5	3	6	11
	103K	M	64		1	3	6	14
	107E	F	64		1	4	7	11
	105G	M	66	0.5	1	4	7	14
	108G	M	66		0.5	2	4	11
	102D	F	78	1	3	5	8	17
	101L	F	78	0.5	1.5	3.5	5.5	13
	106I	M	80	1	3	5	7	13
	105D	M	80	0.5	2	4	5.5	12

Table 34. The Mean and Standard Error of the Mean of Sedimentation Rates of Erythrocytes Expressed in mm. from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

Time	Sex	Basal	Liver Meal	APF
0.5 hr.	M	0.81 ± 0.26	0.46 ± 0.30	0.65 ± 0.25
	F	0.50 ± 0.12	0.44 ± 0.11	0.38 ± 0.13
1 "	M	2.13 ± 0.50	1.33 ± 0.57	1.79 ± 0.48
	F	1.25 ± 0.20	1.36 ± 0.18	1.31 ± 0.21
2 "	M	4.75 ± 0.71	3.08 ± 0.82	4.24 ± 0.70
	F	3.68 ± 0.63	3.28 ± 0.56	3.38 ± 0.66
3 "	M	8.63 ± 1.11	5.50 ± 1.29	7.35 ± 1.08
	F	6.89 ± 1.02	6.17 ± 0.90	5.85 ± 1.12
6 "	M	16.81 ± 1.72	12.50 ± 1.99	15.38 ± 1.67
	F	15.43 ± 1.91	13.78 ± 1.68	12.62 ± 1.98

The analyses of variance of the sedimentation rates of erythrocytes from male and female chicks are as follows:

Completed Analysis of Variance for Male Chicks at 0.5 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.863	0.4315
Pens treated alike	6	6.440	1.0733*
Individuals	36	14.608	0.4058
Total	44	21.911	

Completed Analysis of Variance for Female Chicks at 0.5 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.090	0.045
Pens treated alike	6	1.277	0.213
Individuals	36	6.744	1.873
Total	44	8.111	

Completed Analysis of Variance for Male Chicks at 1 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	4.298	2.149
Pens treated alike	6	23.772	3.962
Individuals	36	71.675	1.991
Total	44	99.745	

Completed Analysis of Variance for Female Chicks at 1 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.097	0.0485
Pens treated alike	6	3.422	0.5703
Individuals	36	29.625	0.8229
Total	44	33.144	

Completed Analysis of Variance for Male Chicks at 2 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	19.46	9.73
Pens treated alike	6	48.52	8.09
Individuals	36	226.46	6.29
Total	44	294.44	

Completed Analysis of Variance for Female Chicks at 2 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	1.31	0.655
Pens treated alike	6	33.87	5.645*
Individuals	36	78.62	2.184
Total	44	113.80	

Completed Analysis of Variance for Male Chicks at 3 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	67.00	33.50
Pens treated alike	6	118.75	19.79
Individuals	36	793.89	22.05
Total	44	979.64	

Completed Analysis of Variance for Female Chicks at 3 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	7.92	3.96
Pens treated alike	6	87.28	14.55
Individuals	36	286.00	7.94
Total	44	381.20	

Completed Analysis of Variance for Male Chicks at 6 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	129.38	64.69
Pens treated alike	6	284.12	47.35
Individuals	36	1,860.08	51.67
Total	44	2,273.58	

Completed Analysis of Variance for Female Chicks at 6 hr.

S.V.	D.F.	S.S.	M.S.
Treatments	2	54.37	27.185
Pens treated alike	6	305.63	50.938
Individuals	36	890.00	24.722
Total	44	1250.00	

From the above analyses, there is no evidence of treatment effects. Environmental effects were present in the sedimentation rates of erythrocytes of male chicks at one-half hour and at two hours for female chicks. The differences between the means (Table 34) were less than the L.S.D.'s. The sedimentation rates can, therefore, be considered as coming from the same population.

The mean and standard error of the mean of the sedimentation rates for erythrocytes of chicks for age 1 and age 2 (Tables 31, 32, and 33) are shown in Table 35. The

Table 35. The Mean and Standard Error of the Mean of Sedimentation Rates of Erythrocytes Expressed in mm. from Chicks of Two Different Ages

Time	Age 1	Age 2
0.5 hr.	0.65 ± 0.11	0.58 ± 0.11
1.0 "	1.61 ± 0.17	1.57 ± 0.17
2.0 "	3.93 ± 0.29	3.54 ± 0.29
3.0 "	7.42 ± 0.72	5.86 ± 0.72
6.0 "	15.65 ± 1.18	13.00 ± 1.18

analyses of variance used in determining the effect of age on sedimentation rates are as follows:

Analysis of Variance at 0.5 hr.

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	0.0868	0.0868
Treatments (T)	2	0.6736	0.3368
A x T	2	0.2986	0.1493
Experimental error	12	4.8750	0.4063
Sampling error	54	9.3125	0.1725
Total	71	15.2465	

Analysis of Variance at 1 hr.

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	0.0313	0.0313
Treatments (T)	2	1.8611	0.9306
A x T	2	1.0833	0.5416
Experimental error	12	12.0000	1.0000
Sampling error	54	41.1875	0.7627
Total	71	56.1632	

Analysis of Variance at 2 hr.

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	2.7222	2.7222
Treatments (T)	2	18.3403	9.1702
A x T	2	11.0486	5.5243
Experimental error	12	35.7500	2.9792
Sampling error	54	160.6250	2.9745
Total	71	228.4861	

Analysis of Variance at 3 hr.

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	43.5555	43.5555
Treatments (T)	2	56.9236	28.4618
A x T	2	38.7570	19.3785
Experimental error	12	223.7500	18.6458
Sampling error	54	535.6250	9.9190
Total	71	898.6111	

Analysis of Variance at 6 hr.

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	126.6702	126.6702
Treatments (T)	2	114.3820	57.1910
A x T	2	72.6319	36.3160
Experimental error	12	599.4583	49.9549
Sampling error	54	1556.4375	28.8229
Total	71	2469.5799	

These analyses show that there is no significant difference in the sedimentation rates between the two ages of chicks. The differences between the means (Table 35) were less than the L.S.D.'s; therefore, the treatments did not cause a significant change in the sedimentation rates.

Table 36. Hematocrit Readings of Blood Expressed in Per Cent from Chicks Receiving the Basal Ration

	Chick no.	Sex	Age in days	Plasma	Packed leucocytes and thrombocytes	Packed erythrocytes
	101D	M	28	64	1	35
	101G	F	35	71	0	29
	101K	M	35	69	2	29
	104H	F	35	66	1	33
	103L	F	37	70	0.5	29.5
Pen 1	105J	F	37	71	3	26
	108C	M	41	65.5	0.5	34
	109D	M	41	73	1.5	25.5
	106K	M	43	62	2	36
	108K	F	43	67.5	1.5	31
	101D	M	69	69	1	30
	101G	F	69	66	2	32
	101K	M	71	69	2	29
	104H	F	71	66	2	32
	101J	F	45	66	3	31
	104I	M	45	68		32
	102G	M	48	71	2	27
	105C	M	48	70.5	1.5	28
	104L	F	50	66	2	32
Pen 2	107H	M	50	62	2	36
	106E	M	52	70	1	29
	108H	M	52	69	1.5	29.5
	108L	F	55	68	2	30
	109K	F	55	69.5	1.5	29
	101J	F	73	65.5	1.5	33
	104I	M	73	69	2	29
	102G	M	76	67	3	30
	105C	M	76	68	3	29
	102C	F	57	67	2	31
	102E	F	57	68.5	2.5	29
	104D	F	59	72	1	27
	104E	M	59	69	2	29
	106H	F	62	67	1.5	31.5
Pen 3	108I	M	62	71.5	1.5	27
	106F	M	64	66	2	32
	109J	M	64	67	2	31
	109L	M	66	68	2	30
	109G	F	66	69	3	28
	102C	F	78	72.5	1.5	26
	102E	F	78	64.5	3.5	32
	104D	F	80	65	2	33
	104E	M	80	66	4	30

Table 37. Hematocrit Readings of Blood Expressed in Per Cent from Chicks Receiving the Liver Meal Ration

	Chick no.	Sex	Age in days	Plasma	Packed leucocytes and thrombocytes	Packed erythrocytes
	101F	F	28	66	2	32
	103I	F	35	70	0	30
	105I	F	35	69	2	29
	103G	F	35	67	0	33
	105H	F	37	71.5	1.5	27
Pen 4	105E	M	37	68	1.5	30.5
	106J	M	41	67	2	31
	107L	M	41	70.5	0.5	29
	108F	M	43	63.5	3.5	33
	106G	M	43	67	2	31
	101F	F	69	67	2	31
	103I	F	69	66.5	1.5	32
	105I	F	71	67	2	31
	103G	F	71	69	0.5	30.5
	102F	M	45	70	2	28
	102J	F	45	68	1	31
	102H	F	48	68	2	30
	104C	F	48	68	2	30
	104J	M	50	65	2	33
Pen 5	106L	F	50	55	3	42
	107J	F	52	69	1.5	29.5
	107F	F	52	65	1.5	33.5
	107K	F	55	66	1.5	32.5
	108E	F	55	66	2	32
	102F	M	73	70.5	1.5	28
	102J	F	73	69.5	1.5	29
	102H	F	76	62	3	35
	104C	F	76	65	3	32
	103D	F	57	69	2	29
	101E	F	57	67	2	31
	103H	M	59	67	4	29
	104F	M	59	72	1	27
	107C	F	62	65	1	34
Pen 6	108D	M	62	72	1	27
	105F	M	64	65	2	33
	105K	F	64	63	2	35
	109H	M	66	64	3.5	32.5
	109E	F	66	69	3	28
	103D	F	78	70	2	28
	101E	F	78	72	2	26
	103H	M	80	69.5	2.5	28
	104F	M	80	70.5	1.5	28

Table 38. Hematocrit Readings of Blood Expressed in Per Cent from Chicks Receiving the APF Ration

	Chick no.	Sex	Age in days	Plasma	Packed leucocytes and thrombocytes	Packed erythrocytes
	101C	M	28	64	1	35
	102I	M	28	66	1	33
	102L	F	35	70	1	29
	103C	M	35	64	0	36
	106D	M	37	73	1.5	25.5
Pen 7	105L	F	37	71	3	26
	102K	M	41	68.5	1.5	30
	104K	F	41	66	5	29
	104G	F	43	68	2.5	29.5
	109I	F	43	67	2	31
	101C	M	69	67.5	1.5	31
	102I	M	69	67.5	1.5	31
	102L	F	71	69	3	28
	103C	M	71	67	2.5	30.5
	101I	M	45	65	4	31
	103F	M	45	66	1	33
	101H	M	48	69	2	29
	103E	M	48	66	2	32
	103J	F	50	71	2	27
Pen 8	107D	F	50	68	2	30
	109C	M	52	72	1	27
	108J	F	52	69	1.5	29.5
	109F	F	55	67.5	1.5	31
	107G	M	55	69	1	30
	101I	M	73	68	1.5	30.5
	103F	M	73	66.5	1	32.5
	101H	M	76	64	4	32
	103E	M	76	70	1.5	28.5
	102D	F	57	65	1	34
	101L	F	57	67	2	31
	106I	M	59	71	1	28
	105D	M	59	72	1	27
	106C	F	62	66	1	33
Pen 9	107I	M	62	67.5	1.5	31
	103K	M	64	65	2	33
	107E	F	64	65	3	32
	105G	M	66	68	1.5	30.5
	108G	M	66	63.5	2	34.5
	102D	F	78	70.5	2.5	27
	101L	F	78	66.5	2	31.5
	106I	M	80	72.5	1.5	26
	105D	M	80	67.5	1.5	31

G. Hematocrit Readings

The hematocrit tubes were centrifuged after the sedimentation rates had been determined. The volume percentages of plasma, packed leucocytes and thrombocytes, and packed erythrocytes are recorded in Tables 36, 37, and 38. The mean and standard error of the mean of the hematocrit readings from male and female chicks are shown in Table 39. The hematocrit readings of the four chicks in each pen (Tables 36, 37, and 38) used to check the effect of age on these readings are not included in Table 39. Figure 15 shows the hematocrit tubes in the sedimentation rack after they had been centrifuged. The blood samples were from the chicks 62 days of age (Tables 36, 37 and 38). The buffy coat of packed leucocytes and thrombocytes can be seen on top of the packed erythrocytes.

Table 39. The Mean and Standard Error of the Mean of Hematocrit Readings of Blood Expressed in Per Cent from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

	Sex	Basal	Liver Meal	APF
Packed erythrocytes	M	30.63±0.49	30.33±0.57	30.91±0.48
" "	F	29.79±0.79	31.58±0.70	30.15±0.82
Packed leucocytes and thrombocytes	M	1.53±0.19	2.08±0.21	1.47±0.18
" "	F	1.75±0.28	1.67±0.25	2.12±0.30
Plasma	M	67.84±0.37	67.58±0.43	67.62±0.36
"	F	68.46±0.84	66.75±0.74	67.73±0.87

The completed analyses of variance of the hematocrit readings expressed in per cent are as follows:

Completed Analysis of Variance of Plasma for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	0.600	0.300
Pens treated alike	6	13.069	2.178
Individuals	36	390.975	10.860
Total	44	404.644	

Completed Analysis of Variance of Plasma for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	23.64	11.82
Pens treated alike	6	59.45	9.91
Individuals	36	261.71	7.27
Total	44	344.80	

Completed Analysis of Variance of Packed Leucocytes and Thrombocytes for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	3.025	1.5125
Pens treated alike	6	3.319	0.5532
Individuals	36	26.567	0.7380
Total	44	32.911	

Completed Analysis of Variance of Packed Leucocytes and Thrombocytes for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	2.048	1.024
Pens treated alike	6	6.792	1.132
Individuals	36	30.160	0.838
Total	44	39.000	

Completed Analysis of Variance of Packed Erythrocytes for Male Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	2.37	1.185
Pens treated alike	6	23.31	3.885
Individuals	36	369.73	10.270
Total	44	395.41	

Completed Analysis of Variance of Packed Erythrocytes for Female Chicks

S.V.	D.F.	S.S.	M.S.
Treatments	2	51.50	25.75
Pens treated alike	6	52.96	8.827
Individuals	36	248.71	6.91
Total	44	353.17	

The above analyses demonstrate that treatments and environment did not produce a change in the hematocrit readings. The differences between the means (Table 39) for the three rations were less than the L.S.D.'s. However, it was observed that the differences and L.S.D.'s for packed erythrocytes of female chicks (Table 40) were very close except for the basal and APF rations. By making further calculations, it was found that the difference between the means of packed erythrocytes from female chicks fed the basal and liver meal rations (1.79) was equal to the L.S.D. when a t-value between 6 and 7% was used instead of the usual 5% value. Also, by further calculations, the packed erythrocyte volume of female chicks receiving the

APF ration was found to be different than those fed the liver meal ration at approximately the 15% level. In other words, these differences approached the 5% level of significance.

Table 40. The Least Significant Differences of Packed Erythrocytes from Male and Female Chicks Receiving the Basal, Liver Meal, and APF Rations

Sex	Basal and liver meal		Basal and APF		APF and liver meal	
	Difference	5% L.S.D.	Difference	5% L.S.D.	Difference	5% L.S.D.
Males	0.29	2.48	0.29	2.26	0.58	2.45
Females	1.79	1.90	0.36	2.05	1.43	1.94

The effect of age on the hematocrit readings (Table 36, 37, and 38) is summarized in Table 41.

Table 41. The Mean and Standard Error of the Mean of Hematocrit Readings of Blood Expressed in Per Cent from Chicks of Two Different Ages

	Age 1	Age 2
Plasma	68.00 ± 0.39	67.85 ± 0.39
Packed leucocytes and thrombocytes	1.53 ± 0.17	2.07 ± 0.17
Packed erythrocytes	30.47 ± 0.45	30.08 ± 0.45

The following analyses of variance were calculated to determine the effect of age on the hematocrit readings:

Analysis of Variance for Plasma Readings

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	0.4204	0.4204
Treatments (T)	2	6.7778	3.3889
A x T	2	14.1111	7.0556
Experimental error	12	66.8333	5.5694
Sampling error	54	309.6875	5.7350
Total	71	397.8299	

Analysis of Variance for Packed Leucocyte and Thrombocyte Readings

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	5.2813	5.2813*
Treatments (T)	2	0.4237	0.2118
A x T	2	0.8957	0.4478
Experimental error	12	12.7917	1.0660
Sampling error	54	42.4375	0.7859
Total	71	61.8299	

Analysis of Variance for Packed Erythrocytes

S.V.	D.F.	S.S.	M.S.
Ages (A)	1	2.7222	2.7222
Treatments (T)	2	8.5069	4.2534
A x T	2	12.5903	6.2952
Experimental error	12	86.3750	7.1979
Sampling error	54	261.7500	4.8472
Total	71	371.9444	

The analyses of variance show that a significant difference exists between the packed leucocyte and thrombocyte readings from the two ages of chicks. The difference between the means was 0.54 (Table 41), whereas the L.S.D. was 0.53.

H. Autopsy Findings

The changes observed in the tissues and organs of the 18 chicks autopsied at the close of the experiment were confined primarily to the hearts, gizzards, and ceca. No apparent symptoms were observed with these tissue changes. The balance of the tissues appeared normal.

Grossly, a majority of the chicks' hearts had diffuse areas of grayish discoloration in the muscle adjacent to the epicardium. Microscopic examination of sections made of the heart muscle failed to reveal an excessive amount of connective tissue or the presence of necrosed muscle fibers. Some subepicardial areas appeared to be thickened with an areolar-type of connective tissue joining the epicardium to the normal heart muscle fibers.

Eight gizzards from the 18 chicks autopsied were observed as having erosions varying from one to eight millimeters in diameter. These areas were localized in all cases. The affected chicks fed the basal ration were 103L, 104E, 101J, and 102G; liver meal ration 109E, 102F, and 105E; and APF ration 103F. Microscopic examination of sections made of the affected gizzards did not show pathologic changes of the mucous membrane. The glands in the tunica propria appeared normal. Apparently these changes were confined to the keratinized epithelial layer.

The ceca of the 18 chicks appeared to be extremely long. The ceca of the smallest chicks measured seven inches in length while those of the largest chicks measured a maximum of 11 inches. The cecal contents appeared normal. No hemorrhages were seen on the mucosa. Microscopic examination of the cecal and rectal contents failed to demonstrate the presence of internal parasites.

Chick 106J, fed the liver meal ration, was mentioned above as losing weight the last two weeks of the experiment (Table 7). The chick had become thin and emaciated. On autopsy a mass of tissue, 4 x 10 x 12 cm., was found in the abdominal cavity, dorsal to the intestine, largely to the right of the median plane, and replacing the anterior lobe of the right kidney. This tissue was gray in color, solid consistency, easily torn by applying pressure, cut easily, and appeared gray on the cut surface. Figure 16 shows that this mass of tissue was an embryonal nephroma. Both lungs were edematous and showed three areas of pneumonia five to seven millimeters in diameter.

I. Polarized Light

Various degrees of degeneration were observed in the myelin sheaths of the sciatic nerves between crossed prisms of the polarizing microscope (Figures 17 through 25).

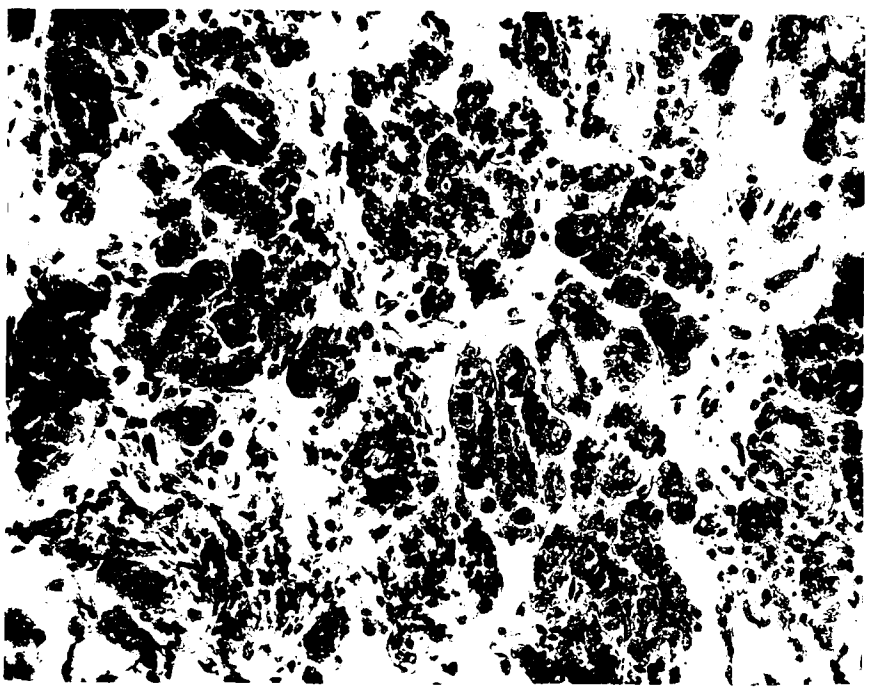
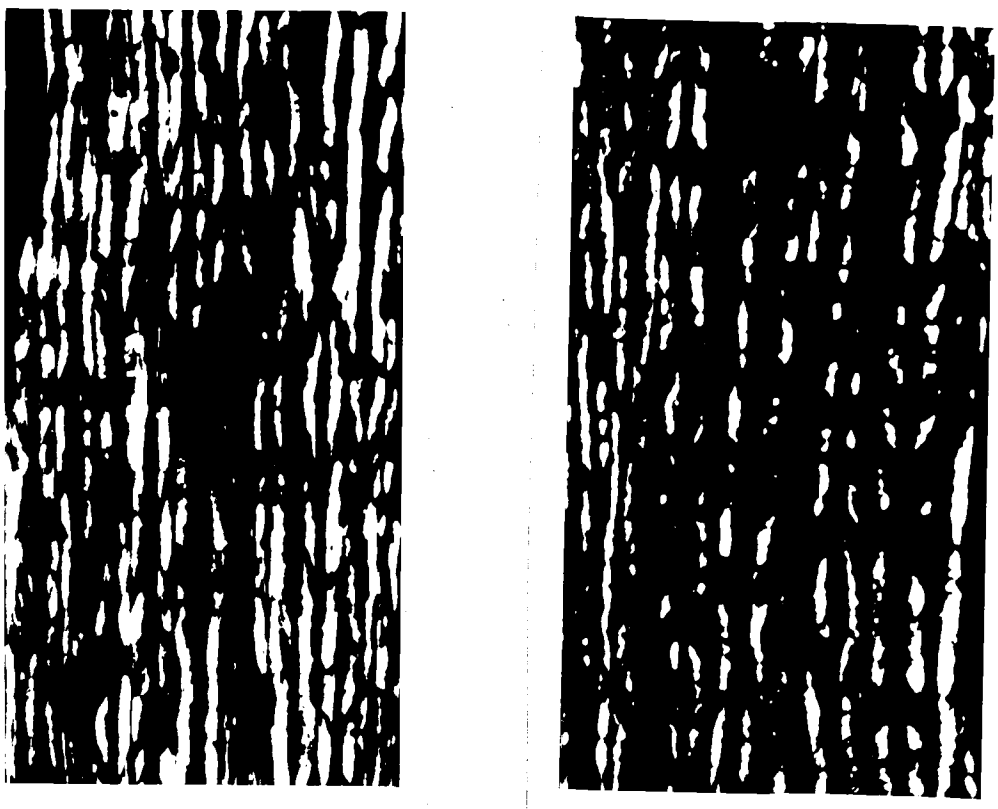


Figure 16. Embryonal nephroma found in the abdominal cavity of chick 106J. Hematoxylin and triosin. X 300.

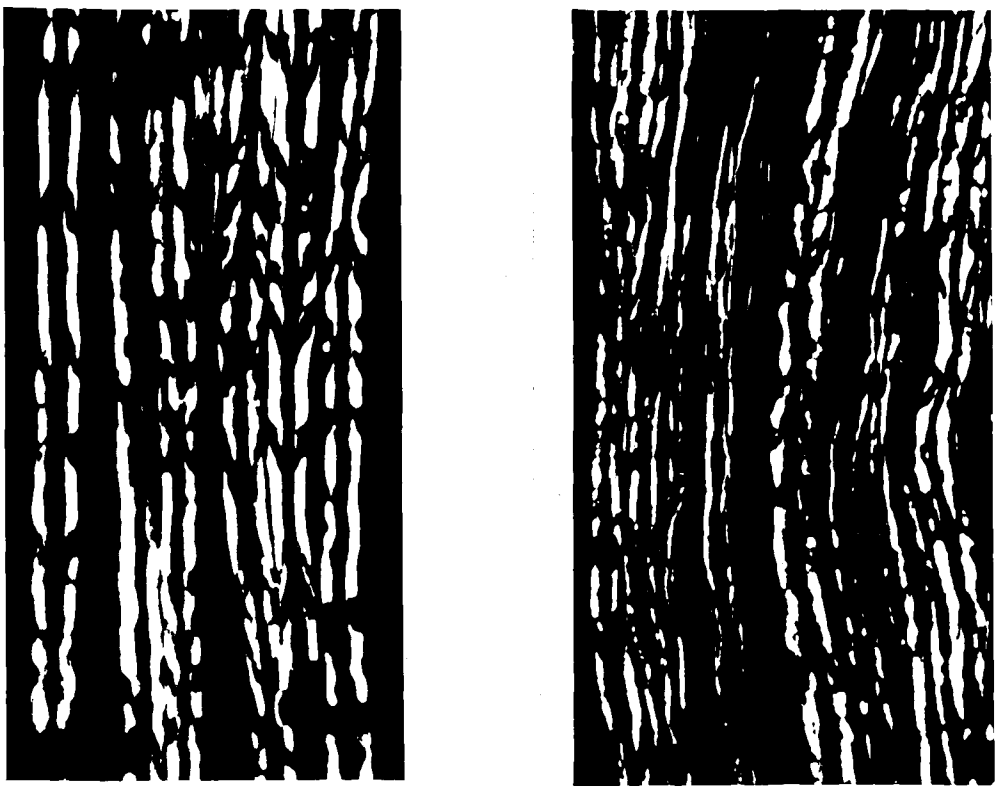
Chick	109G (female)	101J (female)
Wt. gain	791 gm.	1,293 gm.

Figure 17. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the basal ration. X500.



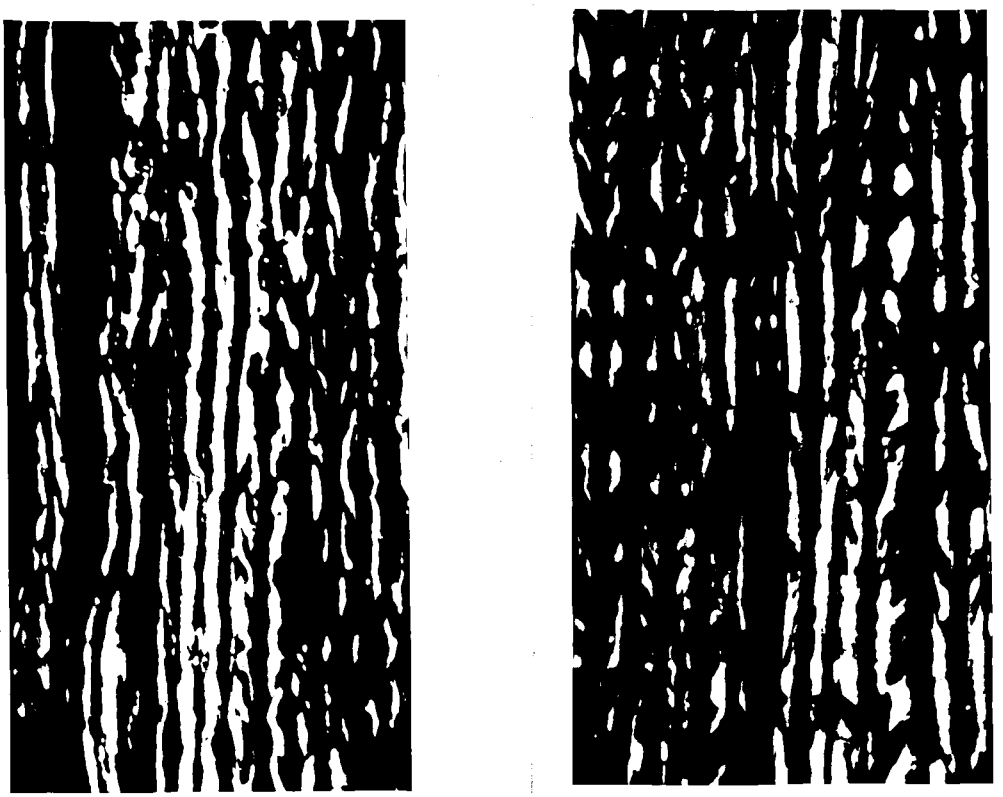
Chick	103L (female)	104E (male)
Wt. gain	1,449 gm.	1,549 gm.

Figure 18. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the basal ration. X500.



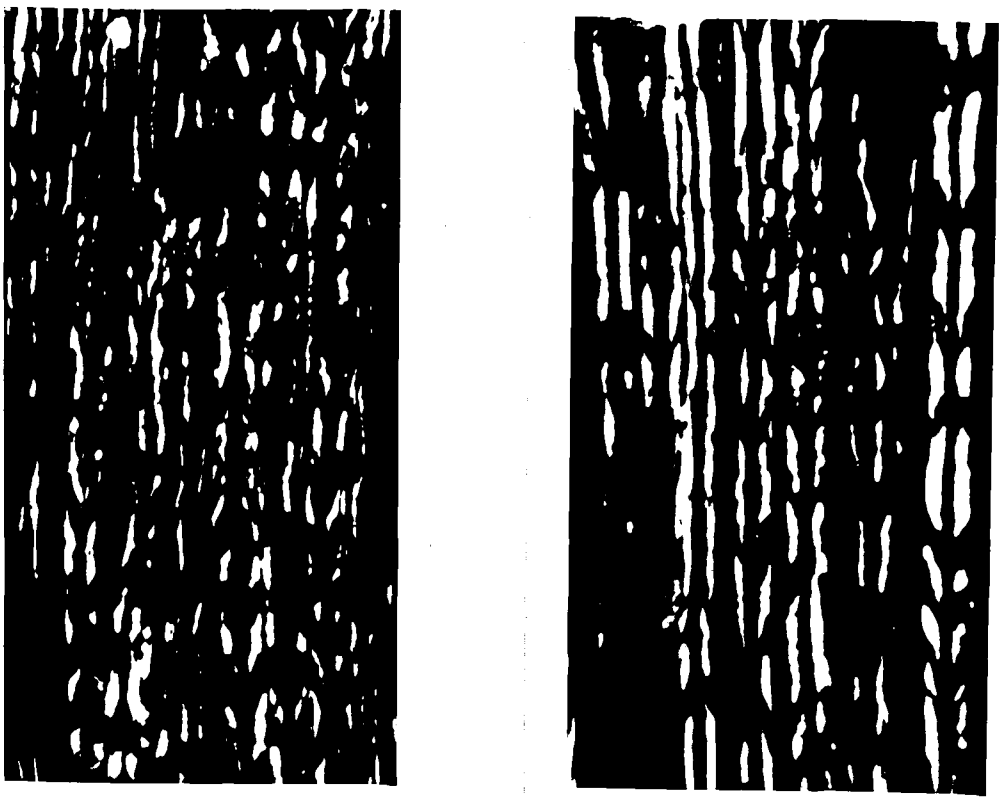
Chick	102G (male)	101K (male)
Wt. gain	1,716 gm.	1,988 gm.

Figure 19. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the basal ration. X500.



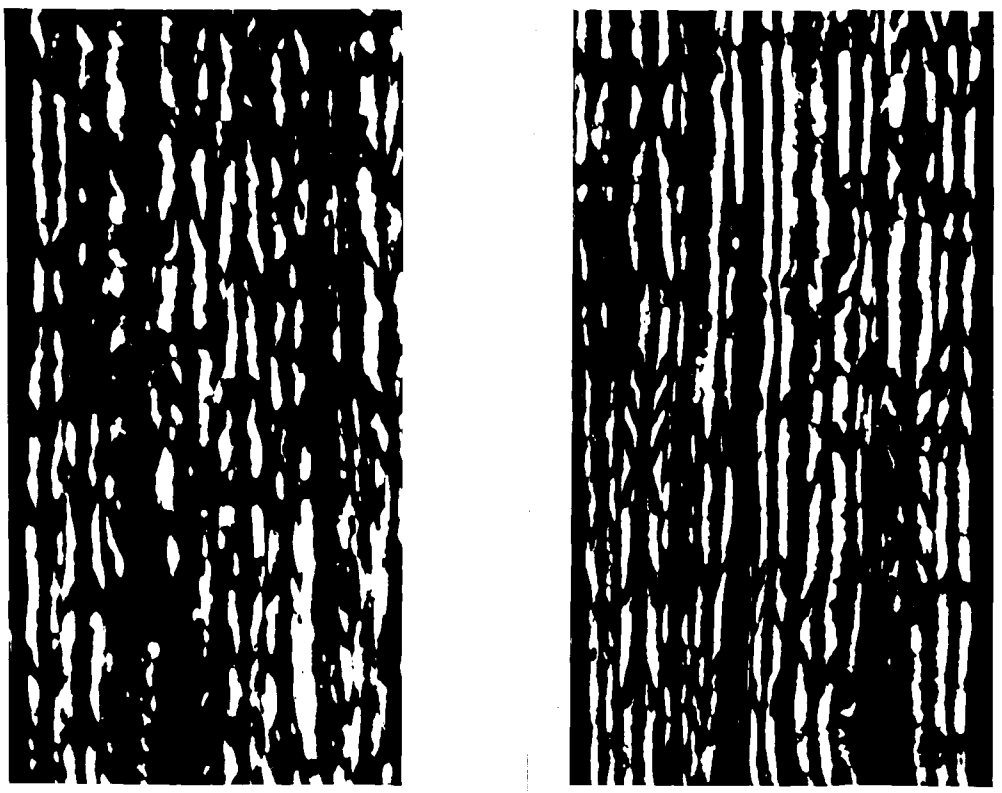
Chick	102J (female)	101F (female)
Wt. gain	1,272 gm.	1,311 gm.

Figure 20. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the liver meal ration. X500.



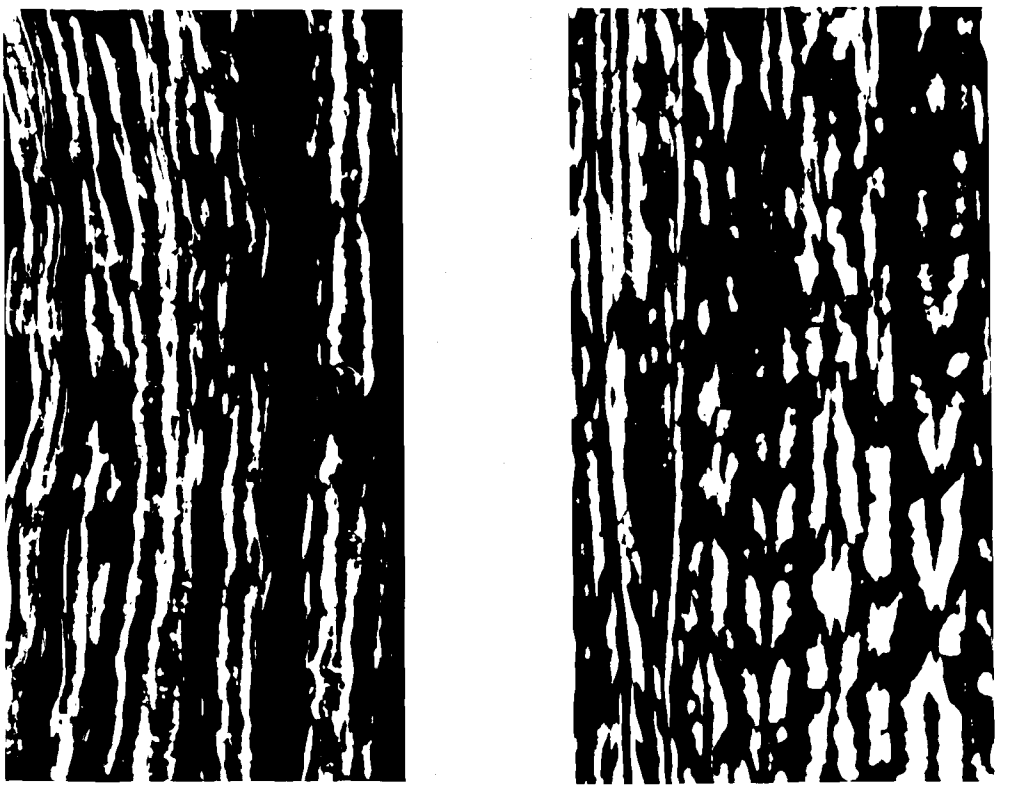
Chick	109E (female)	105E (male)
Wt. gain	1,603 gm.	1,800 gm.

Figure 21. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the liver meal ration. X500.



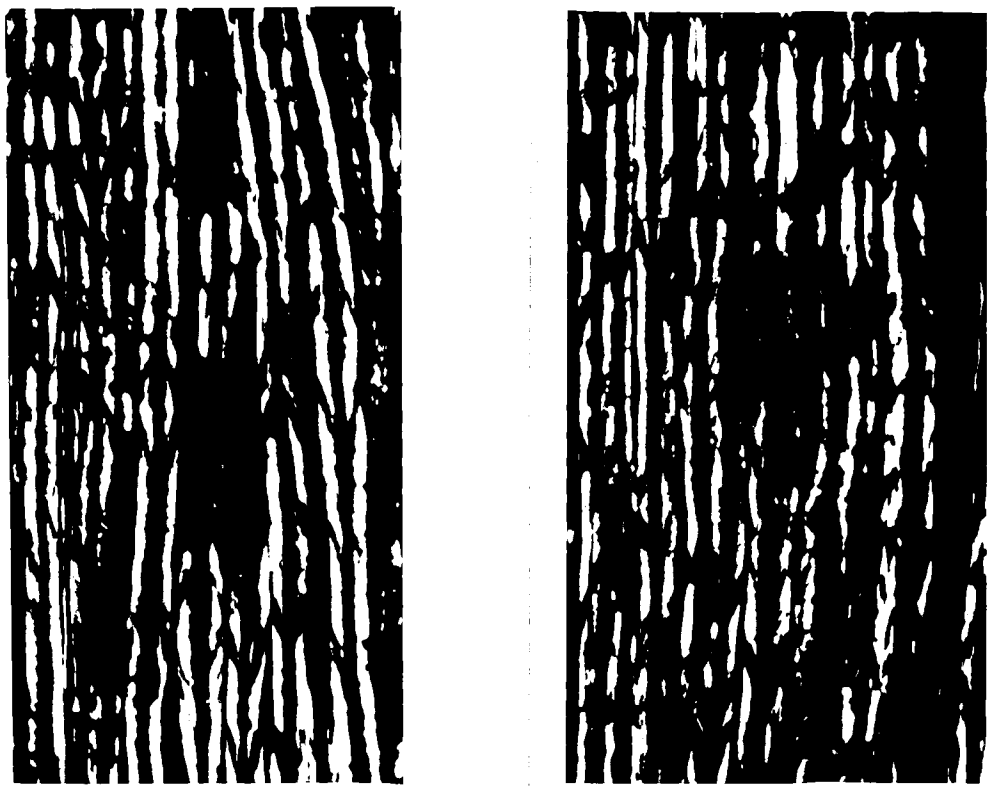
Chick	102F (male)	104F (male)
Wt. gain	1,996 gm.	2,055 gm.

Figure 22. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the liver meal ration. X500.



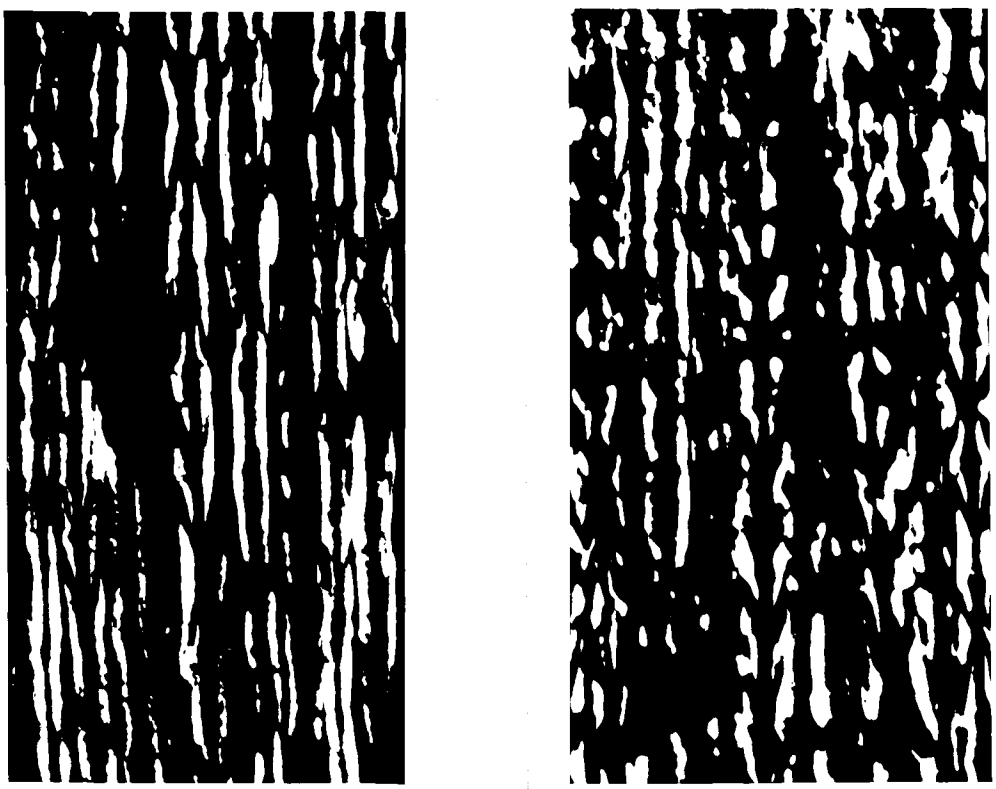
Chick	108J (female)	102L (female)
Wt. gain	1,195 gm.	1,401 gm.

Figure 23. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the APF ration. X500.



Chick	103F (male)	101L (female)
Wt. gain	1,454 gm.	1,546 gm.

Figure 24. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the APF ration. X500.



Chick	101C (male)	106I (male)
Wt. gain	1,723 gm.	2,065 gm.

Figure 25. Photomicrographs taken between crossed prisms of a polarizing microscope of sciatic nerves from chicks fed the APF ration. X500.

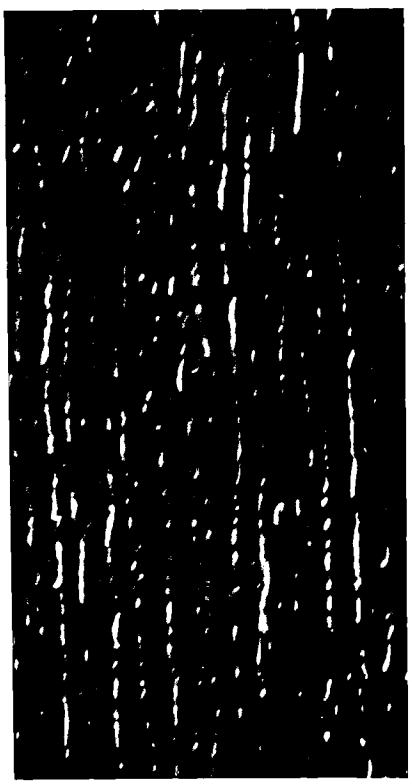


Figure 26. Photomicrograph taken between uncrossed prisms of a polarizing microscope of the sciatic nerve from chick 101L shown in Figure 24. X500.

By checking the six photomicrographs of the sciatic nerves of chicks fed one ration with those of chicks fed another ration, it was difficult to determine if the degenerative changes were more severe in one group of chicks than another. Therefore, Table 42 was made in order to evaluate the observed changes and arrive at more accurate conclusions.

Table 42. Ratings of the Degenerative Changes Observed in the Myelin Sheaths of Sciatic Nerves Between Crossed Prisms of a Polarizing Microscope of Chicks Fed the Three Rations

Chicks fed basal		Chicks fed liver meal		Chicks fed APF	
109G	++	102J	+	108J	+
101J	++	101F	++	102L	++
103L	++	109E	+++	103F	+
104E	++	105E	++	101L	+
102G	+	102F	+	101C	+
101K	+	104F	+	106I	+++
Total	10 +		10 +		9 +

Realizing that this method of evaluating the degenerative changes in the myelin sheaths of sciatic nerves is not entirely satisfactory, it should serve, however, in determining whether or not the degenerative changes were more severe in the chicks fed one ration as compared with another ration. The sciatic nerves of all the chicks show some myelin sheath degeneration (Figures 17 through 25 and Table 42), and the treatments (rations) did not cause an appreciable difference

in the degree of myelin sheath degeneration.

The sciatic nerves of chicks 109E (Figure 21) and 106I (Figure 25) revealed the most severe myelin sheath degeneration while chick 104F (Figure 22) the least degeneration. The swelling of myelin sheaths in chicks 103F (Figure 24) and 102G (Figure 19) indicates a degenerative change which may eventually lead to severe degeneration as illustrated in chick 106I (Figure 25). The position of the longitudinal section in relation to the axis cylinder of the nerve fiber may alter the general appearance, especially in the width of the anisotropic material (white) as shown in the photomicrographs. The nodes of Ranvier and the incisures of Schmidt-Lantermann in the myelin sheaths appear isotropic (black) in the photomicrographs and must not be confused with the degenerative areas in the myelin sheaths.

Figure 26 demonstrates the need of crossed prisms in a polarizing microscope to detect degeneration of myelin sheaths. The photomicrograph of chick 101L in Figure 26 taken between uncrossed prisms corresponds to that of chick 101L in Figure 24, the latter photomicrograph being taken between crossed prisms.

J. Marchi Method

The results of the Marchi method of staining tissues are summarized in Table 43. These observations are not

Table 43. Results of the Marchi Staining Method

	Chick no.	Sex	Wt. gain gm.	Brain medulla	Cerebellum	Spinal cord	Sciatic nerve	Skeletal muscle	Heart	Liver	Kidney	Spleen	Pancreas	Proventriculus	Gizzard	Small intestine	Cecum	Rectum	Testis	Ovary	Thyroid	Thymus	Lung
	109G	F	791	T	T	T	T	-	-	+	-	-	T	T	-	T	-	T	-	-	-	T	-
	101J	F	1,293	T	T	T	T	-	T	T	T	-	T	T	-	T	T	T	-	-	-	T	-
Basal	103L	F	1,449	T	T	+	+	-	T	-	T	-	T	T	-	T	-	T	-	-	-	T	-
ration	104E	M	1,549	+	+	+	+	-	T	T	T	T	+	T	-	+	-	-	T	-	-	T	-
	102C	M	1,716	+	+	+	T	-	T	T	T	T	T	-	-	-	-	-	T	-	-	T	-
	101K	M	1,988	+	+	+	+	-	T	T	T	-	T	T	-	-	T	T	T	-	-	-	-
	102J	F	1,272	T	T	+	T	-	T	T	-	-	T	T	-	-	T	T	-	-	-	T	-
	101F	F	1,311	+	+	+	+	-	T	T	T	-	T	T	-	T	T	-	-	-	-	T	-
Liver	109E	F	1,603	T	T	T	T	-	T	++	T	-	T	T	-	T	-	T	-	-	-	-	-
meal	105E	M	1,800	+	+	+	+	-	T	+	T	-	+	T	T	T	-	-	T	-	-	T	-
ration	102F	M	1,996	+	+	+	+	-	-	T	T	-	+	T	-	T	T	-	T	-	-	-	-
	104F	M	2,055	+	+	+	+	-	+	T	T	-	T	T	-	T	T	T	T	-	-	T	-
	108J	F	1,195	T	T	T	T	-	-	+	T	-	T	T	-	T	-	T	-	-	-	T	-
	102L	F	1,401	+	+	+	+	-	+	++	T	T	+	T	-	T	T	T	-	-	-	T	-
APF	103F	M	1,454	+	+	++	+	-	T	+	T	-	+	-	-	T	-	-	T	-	-	T	-
ration	101L	F	1,546	T	T	T	T	-	-	++	-	-	T	T	-	-	-	-	-	-	-	T	-
	101C	M	1,723	T	T	T	T	-	T	T	T	-	T	T	-	T	T	T	T	-	-	T	-
	106I	M	2,065	+	+	+	+	-	+	T	T	-	T	T	-	T	T	-	T	-	-	+	-

- signifies no fatty change
T " trace, only enough fatty change for identification
+ " moderate fatty change, less than 25% of area or number affected
++ " marked fatty change, 25 to 50% of area or number affected

necessarily pathologic because osmic acid stains fat wherever it may be present. The observations recorded for the spleen, pancreas, proventriculus, small intestine, cecum, rectum, testis, and thymus are considered normal. The alterations observed in the brain medulla, cerebellum, spinal cord, sciatic nerve, heart, liver, kidney, and gizzard are considered pathologic; however, those changes recorded as traces should probably be considered either normal or of minor importance.

With these limitations in mind, degenerative changes of major importance (+ and ++) are present in the brain medulla, cerebellum, spinal cord, sciatic nerve, heart, and liver. Figure 27 demonstrates these alterations in the myelin sheaths of the spinal cord from chick 102J fed the liver meal ration. The affected myelin sheaths are black, whereas those sheaths appearing lighter in color are considered normal. Figure 28 is a cross section of a sciatic nerve from chick 101K fed the basal ration. A number of the myelin sheaths appear to have undergone a degenerative change. Some sheaths are partly normal while the remainder of these sheaths have become stained with osmic acid. The changes in the central nervous system and sciatic nerves are common to the chicks receiving the three rations.

The fatty change in the liver was most prevalent in chicks receiving the APF ration (Table 43), followed in

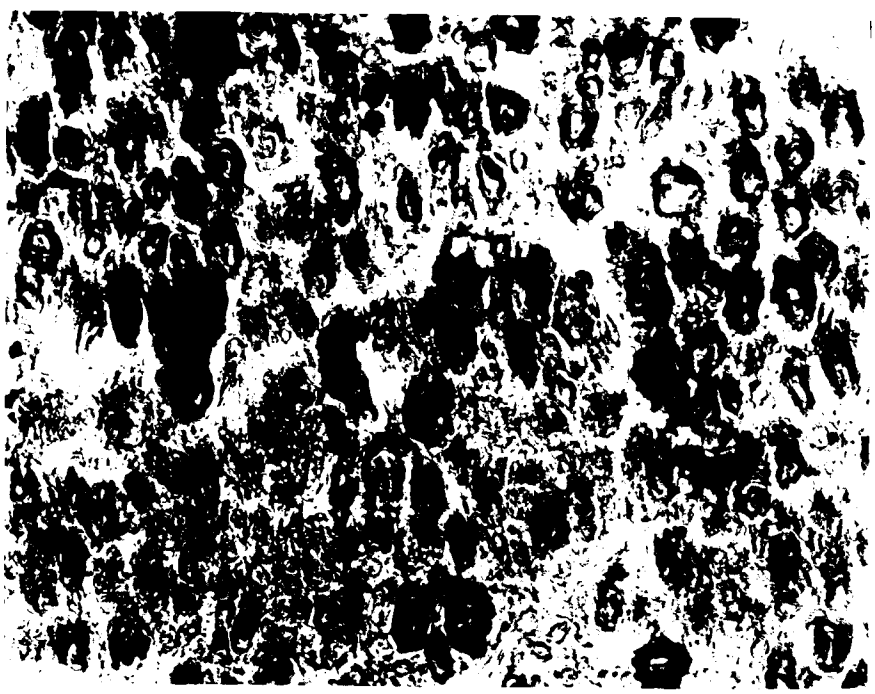


Figure 37. Cross section of the spinal cord from chick 1035 showing myelin sheath degeneration. Marchi. X500.

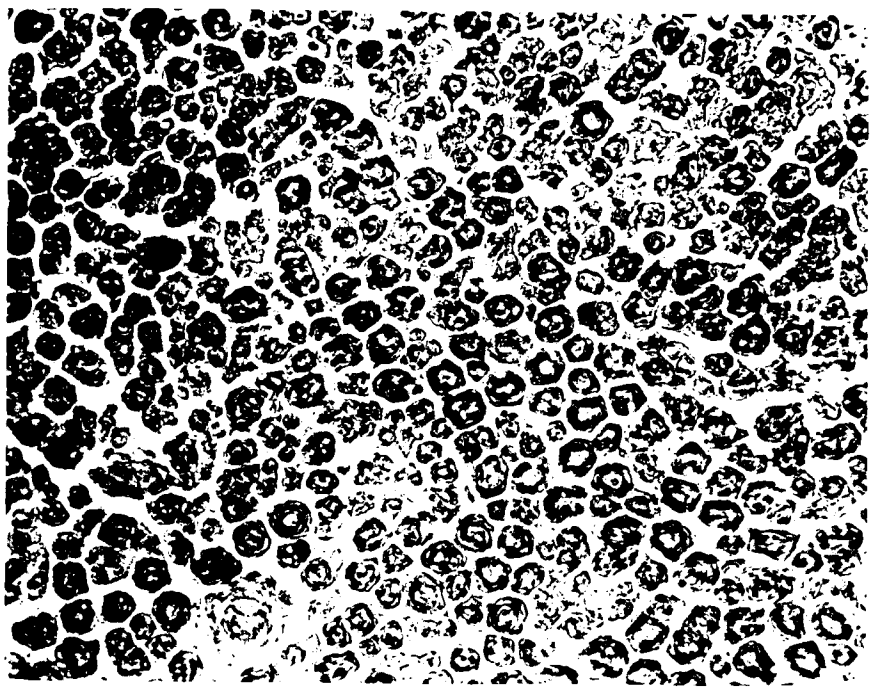


Figure 32. Cross section of a sciatic nerve from chick 101k showing myelin sheath degeneration. Osmic. X500.

order by the liver meal and basal rations. Figure 29 reveals the presence of the fatty change in the hepatic cells of the liver from chick 101L fed the APF ration. This change was not limited to the area around the central vein, as the photomicrograph may suggest, but was uniformly present throughout the liver lobules.

The hearts of three chicks, 104F fed the liver meal ration, 102L and 106I fed the APF ration, revealed black droplets in muscle fibers. This fatty change was present in a large number of fibers.

Figure 30 demonstrates the normal distribution of fat in the ova. Numerous small ova can be seen near the margins of the photomicrograph. It was noted that fat is also present in the interstitial cells of the testis (Table 43) of which Figure 31 is an example. The fat was not observed within the seminiferous tubules. Since the fat was present in the interstitial cells of all the male chicks fed the three rations, it is believed that the presence of fat in this location is normal.

Minute fat droplets were seen in almost every cell of the glandular portion of the pancreas and proventriculus of the affected chicks (Table 43). These droplets were very small and uniform in size. The spleen, thymus, and lymphoid tissue present in the wall of the small intestine, ceca, and rectum were often observed as containing a small

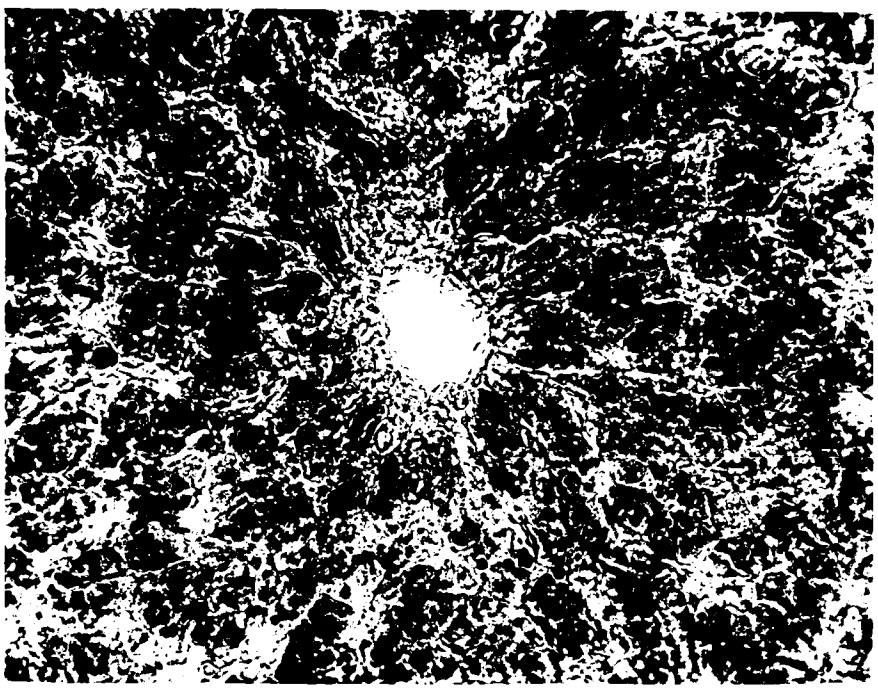


Figure 29. Liver of child 1011 showing a fatty change around a central vein. Marchi. X 500.

Figure 30. Ovary of chick 153L showing distribution of fat in the ova. Parent. X140.

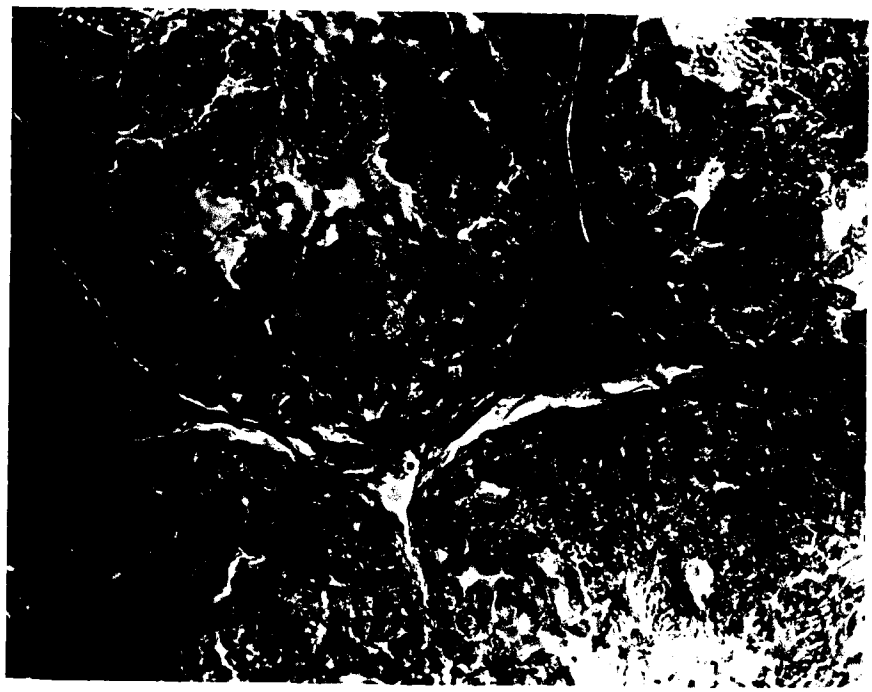


Fig. [illegible]. Cells of chick 105 showing the presence of fat in the interstitial cells. Osmi. X500.

amount of material which stained black with osmic acid. This finding was considered to be normal or at least of no pathologic significance.

K. Hematoxylin and Triosin

Portions of tissue corresponding to those taken from chicks for the Marchi method of staining (Table 43) were stained with hematoxylin and triosin. Pathologic changes were observed in the heart, liver, and kidney. The changes in the heart have been discussed.

A fatty change, indicated by the appearance of clear vacuoles in the hepatic cells, was noted in the livers of chicks (109E, 102L, and 101L) showing a two plus change by the Marchi method (Table 43). Part of the livers from chicks with a one plus or trace showed some evidence of vacuolization in the hepatic cells. These vacuoles were smaller in size and less numerous than those with a two plus change.

The kidneys from the chicks listed in Table 43 showed pathologic changes. Albuminous degeneration was present in many of the kidney tubules. The nuclei of the affected tubule cells were pyknotic in all cases except those in the kidneys of chicks 101C fed the APF ration, 101J and 103L fed the basal ration. Figure 32 demonstrates the

pyknotic nuclei of the tubule cells and also the albuminous degeneration of the cytoplasm of the tubule cells.

Increased cellularity was observed in the center of the capillary tufts of the glomeruli in the kidneys of all the chicks. It is believed that this condition is probably a normal characteristic of chicken kidney.

Hyalinized areas, 10 to 50 microns in diameter, were present in the medulla of the thymus. These areas are distinct from Hassall's corpuscles and yet they appear in the same location in the thymus. Since Hassall's corpuscles may have hyalinized centers, it seems probable that these hyalinized areas may eventually become Hassall's corpuscles.

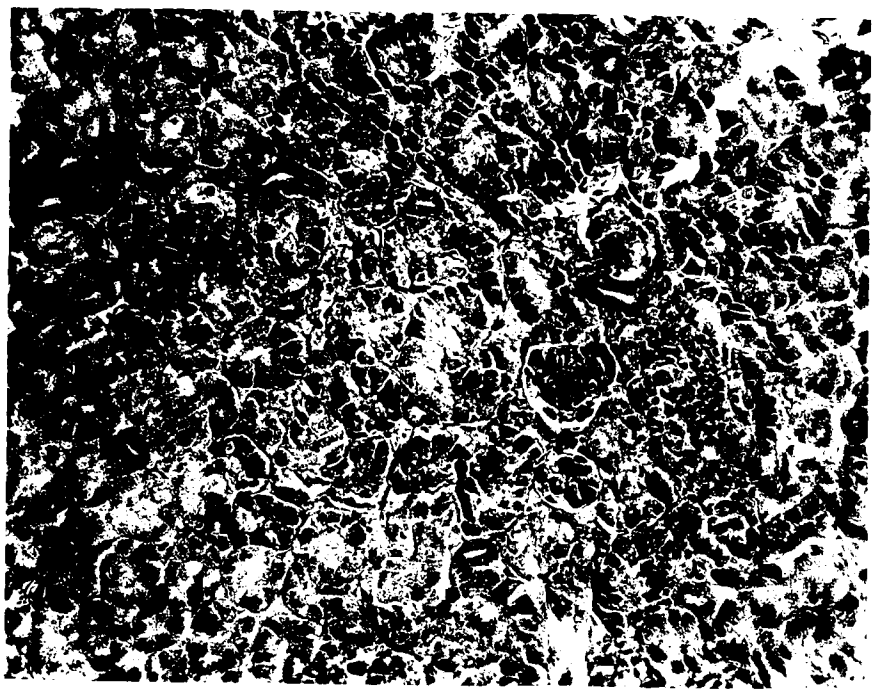


Figure 37. Kidney of calf 161L showing pyknotic nuclei and albuminous degeneration of the tubule cells. Hematoxylin and triosin. X300.

V. DISCUSSION

The chicks used in this experiment were hatched from eggs produced by hens fed a vitamin B_{12}-deficient ration for approximately five months. It was presumed that the vitamin B_{12} content in the tissues of the hens had been reduced and consequently produced eggs of low vitamin B_{12} content. By feeding this ration to the hens, hatchability had decreased although no mortality occurred when the hatched chicks were fed the all-plant protein basal ration. Apparently, the vitamin B_{12} content of the chicks' tissues had not been depleted sufficiently to interfere with the livability of the chicks receiving the basal ration. No deaths occurred in the chicks fed the liver meal and APF rations except chick 104G (Table 10) which died shortly after being bled.

Perhaps a smaller quantity of the APF supplement would have given as good results as the 0.5% which was included in the APF ration. Ott et al. (1948) suggested 30 μg. of vitamin B_{12} per kg. of ration for chicks. Realizing that the vitamin B_{12} content of the APF supplement had been assayed by the Lactobacillus lactis Dorner method, it was decided to include an ample amount of the

APF supplement in the ration since other compounds (Shive et al., 1948b) interfere with this method of assay. By including 0.5% APF supplement in the APF ration, 62.5 μg. of vitamin B_{12} were in each pound of ration according to the L.L.D. assay.

The amino acid requirements of chicks as suggested by Almquist (1947) and the National Research Council (1946) were not met in all cases (Tables 1, 2, and 3); however, if the amino acid content of wheat midds had been included in the totals most of the requirements would probably be fulfilled. A satisfactory reference of the amino acid content of wheat midds was not found.

No apparent dietary cause can be found for the perosis occurring in six of the 90 chicks. The requirements for choline, manganese, biotin, inositol, and folic acid appeared to be fulfilled. Excessive calcium, phosphorus, and iron were not present in the ration to precipitate the manganese. The wire floors may be a contributing factor to the perosis although the existence of perosis in male chicks only may suggest a dietary factor since the male chicks grew more rapidly than the females (Figures 9, 10, and 11). The gains in weight at two-week intervals show that the females deviated from the means less than the males. This accounts for the lower standard errors of the means calculated for the females in comparison with the males (Table 14).

Female chicks were found to be more satisfactory statistically than males in obtaining significant growth responses among the rations (Figure 12). On the other hand, male chicks showed more environmental effects statistically than female chicks (Figure 13). The male and female chicks were subjected to the same environmental conditions because no sexing was done and both sexes were present in all pens. The larger standard errors of the means for weight gains in male chicks as compared with those of female chicks account for the treatment effects not being significant statistically (Figure 12).

Figure 14 indicates that feed efficiency was the best in chicks fed the liver meal ration. The liver meal ration also promoted the best growth in chicks. Apparently, some nutrients are lacking or not present in sufficient quantity in the APF ration since the feed efficiency from this ration was the poorest of the three rations after eight weeks. No attempt was made to weigh back the feed wasted which had fallen on the trays below. No difference was observed in the amount of feed wasted among the pens.

The method used in determining hemoglobin corrected the higher readings usually obtained from the nucleated erythrocytes of chicken blood. The method described by Schultze and Elvehjem (1934) was employed with certain modifications enabling the use of the Klett-Summerson

photoelectric colorimeter. The significant difference observed between the hemoglobin levels of female chicks fed the basal and liver meal rations (Table 21) was also found to be reflected in the packed erythrocyte volume (Tables 39 and 40). The latter difference approached the 5% level of significance whereas the differences between the other rations were not as great.

The absence of a significant interaction (A x T) was observed in the hemoglobin values when the effect of age was determined. In all subsequent blood studies in which the effect of age was considered, interaction was not significant. In the event that interaction had been significant, it would mean that the treatments behaved differently or caused various reactions with the different age groups.

Wiseman's method of counting erythrocytes and leucocytes was used because Olson (1935) found this method to be the most satisfactory for counting erythrocytes. Since vitamin B_{12} is known to have an erythropoietic function, the method most apt to detect smaller differences in the erythrocyte counts was chosen.

Corrections in the sedimentation rates of erythrocytes according to volumes of packed erythrocytes were not made. Apparently, a chart correcting the sedimentation rate of chicken erythrocytes according to volume of packed erythrocytes is not available. The chart used in correcting the

sedimentation rate of human erythrocytes (Wintrobe, 1946) may be applicable to other species of mammalian blood; however, it was not considered practical for chicken blood. A project checking the efficacy of the chart correcting the sedimentation rate of human erythrocytes when applied to chicken blood may prove that it can be used with reasonable accuracy. The sedimentation rates of chicken erythrocytes should, however, be corrected according to the packed erythrocyte volumes before accurate conclusions can be made. In this experiment no significant difference existed between the sedimentation rates of the erythrocytes from chicks fed the three rations at the various time intervals; therefore, corrections in the sedimentation rates would probably not make a significant difference in these observations. The difference in packed erythrocyte volume of female chicks receiving the basal and liver meal rations as discussed above, would cause some change in the corrected sedimentation rates, but whether or not the differences would be significant can only be determined by statistical analysis of the corrected values.

Table 41 shows that the difference between the means of packed leucocytes and thrombocytes produced by age is 0.54. This difference was found to be statistically significant. After considering that the readings of this

small layer of packed leucocytes and thrombocytes (Figure 15) were made by visual observation and the difficulty in making readings less than 0.5 mm. in a hematocrit tube, it appears that error in reading may have caused the significant difference. Age did not cause a difference in the total leucocyte counts; therefore, it is unlikely that the volume of packed leucocytes and thrombocytes should be significant unless a difference in the number of thrombocytes existed. The latter was not determined. After taking these factors into consideration, it is concluded that a significant difference in the volume of packed leucocytes and thrombocytes was not produced with age.

The gizzard erosions observed in eight of the 18 chicks autopsied were limited to the keratinized layer of the epithelium. The glands in the tunica propria of the mucous membrane were not involved. It appears that the erosions were almost healed. It is believed that these erosions were probably present in the chicks at hatching time or shortly thereafter since the lesions had almost disappeared. Large ceca were present in the chicks varying from seven to 11 inches in length. Sisson and Grossman (1938) state that the ceca of the chicken are about seven inches long. Lippincott and Card (1939) state that the ceca are from four to six inches in length. It is difficult to say what length is normal or abnormal. Perhaps the ceca observed

as being longer than usual in this investigation may be due to the type of ration, confinement of chicks, and other unknown factors. The fiber content of the rations was not excessive, being slightly less than 6%. Microscopic examination of the sections made of the ceca failed to reveal pathologic changes.

The leg weakness observed in chicks fed the basal and APF rations was not correlated with myelin sheath degeneration because chicks fed the liver meal ration showed as much myelin sheath degeneration as those receiving the other rations. The Marchi method checked closely with the observations made with the polarizing microscope between crossed prisms at the point of greatest birefringence. Figures 17 through 25 taken with the polarizing microscope show slightly more myelin sheath degeneration of the sciatic nerves than those stained by the Marchi method. Figure 28 is an example of the latter method.

The presence of the fatty change observed in the livers (Table 43 and Figure 29) and also the degenerative changes of the kidney tubules (pyknotic nuclei and albuminous degeneration) of nearly all the chicks listed in Table 43 suggest a choline deficiency. These alterations conform to the described pathologic changes in the liver and kidney (Follis, 1948) occurring in choline deficiency of mammals. The occurrence of perosis in six of the 90

chicks is further evidence that the rations may have been deficient in choline. The amount of choline in the rations, however, exceeded the recommended quantity suggested by the National Research Council (1946).

Kilborn (1939) found that ducks and pigeons failed to show a significant increase in the fat content of livers by feeding a choline-free, high fat diet. Kilborn concluded that birds differed from mammals in fat metabolism. Fasting, fasting plus anterior pituitary injections, or choline-deficient diets did not cause a significant increase in the fat content of livers in chickens, whereas such treatments are known to cause a significant increase in the liver fat of mammals. In this report microscopic examination of the tissues was not mentioned. The duration of the experiment for ducks and pigeons varied from 20 to 33 days. The fat in the liver of chickens was checked from two to five days after the anterior pituitary injections were made. It appears that the conclusion drawn from this experiment that excess fat is not deposited in the livers of chickens fed choline-deficient rations may not be entirely satisfactory.

The minute black droplets seen in the enzyme-secreting cells of the pancreas and proventriculus stained by the Marchi method (Table 43) are probably of no pathologic importance. The time interval between feeding and killing

of the chicks may account for the slight variation in results. The chicks were self-fed thereby having feed in front of them at all times. The time of killing did vary because one chick was autopsied at a time and only three chicks were killed in a day because it was necessary to secure the tissues immediately after death for microscopic study.

VI. SUMMARY AND CONCLUSIONS

1. Treatments (rations) produced a highly significant difference statistically in the weight gains of the 45 female chicks for the first 10 weeks of the experiment and a significant difference at 12 weeks of age.

2. Treatments produced a significant difference statistically in the weight gains of the 45 male chicks for the first two weeks of the experiment and no effect on the weight gains during the balance of the experiment.

3. A highly significant difference was present between the weight gains of female chicks fed the basal and liver meal rations at 2, 4, 6, 8, 10, and 12 weeks of age, whereas this difference existed only at two weeks of age in male chicks.

4. A significant difference was present between the weight gains of male chicks fed the basal and liver meal rations at four and eight weeks of age.

5. A highly significant difference existed between the weight gains of female chicks fed the basal and APF rations at four and six weeks of age while a significant difference occurred between the weight gains of female chicks at 2, 8, and 10 weeks of age and at two weeks of age for male chicks.

6. Female chicks did not gain as rapidly as male chicks but the weight gains deviated from the means less than the male chicks.

7. The standard errors of the mean of weight gains were consistently larger for male chicks than females, indicating the influence of environmental effects.

8. Environmental effects produced a highly significant difference in the weight gains of male chicks at four, six, and eight weeks of age and a significant difference in the weight gains at 10 weeks, whereas no significant environmental effects were present in the weight gains of female chicks.

9. This experiment indicates that female chicks should be a better assay animal for vitamin B_{12} than male chicks due to the smaller standard error of the means obtained from the weight gains and the absence of significant environmental effects.

10. The feed efficiency of the chicks fed the liver meal ration was the best throughout the experiment followed in order by those fed the APF and basal rations to eight weeks of age with the group fed the APF ration being the poorest thereafter.

11. A highly significant difference was present between the feed efficiency of chicks fed the basal and liver meal rations and also between those fed the APF and liver

meal rations while a significant difference in feed efficiency did not exist between the chicks fed the basal and APF rations.

12. A significant difference was found between the hemoglobin levels of the female chicks fed the basal and liver meal rations while no significant difference existed between the hemoglobin levels of chicks fed the basal and APF rations and also between those fed the liver meal and APF rations.

13. The hemoglobin levels of the male chicks fed the three rations were not significantly different.

14. The packed erythrocyte volume of female chicks fed the basal and liver meal rations approached the 5% level of significance.

15. Treatment effects on erythrocyte counts, total leucocyte counts, differential counts, sedimentation rates, plasma volume, and packed leucocytes and thrombocytes were not significant.

16. Environmental effects produced a highly significant difference in the basophil counts of male chicks, a significant difference in the sedimentation rate of erythrocytes of male chicks in one-half hour, and in female chicks a significant difference in the total leucocyte counts, lymphocyte counts, and sedimentation rate of erythrocytes in two hours.

17. Age (21 to 37 days) produced a highly significant difference in the hemoglobin levels of chicks and a significant difference in the eosinophil counts, whereas the remaining blood determinations had not changed significantly.

18. Eight gizzards from the 18 chicks autopsied were observed as having erosions in the keratinized portion of the epithelium while the glands in the tunica propria of the mucous membrane appeared normal microscopically.

19. Grossly, a majority of the hearts showed areas of grayish discoloration in the muscle under the epicardium which on microscopic examination appeared to be an areolar-type of connective tissue joining the epicardium to the normal muscle fibers. This condition was attributed to the needle puncturing the heart in obtaining blood samples.

20. The ceca of the chicks were observed as being unusually long varying from seven to 11 inches in length but showing no pathologic changes microscopically.

21. The leg weakness of chicks fed the basal and APF rations was not associated with myelin sheath degeneration.

22. Treatments produced no appreciable change in the degree of myelin sheath degeneration in sciatic nerves of chicks fed the three rations as observed between crossed prisms of a polarizing microscope at the point of greatest birefringence and by the Marchi method of staining.

23. The Marchi method of staining and the

hematoxylin-triosin stain revealed a fatty change in the liver, the greatest change appearing in the livers of chicks fed the APF ration followed in order by those fed the liver meal and basal rations.

24. The hearts from two chicks fed the APF rations and one chick fed the liver meal ration showed a fatty change by the Marchi method while a majority of the other chicks fed the three rations showed only a trace of fatty change in the heart muscle fibers which was considered to be of no pathologic importance.

25. The Marchi method of staining revealed the presence of fat in ova, interstitial cells of testis, and in the enzyme-secreting cells of the pancreas and proventriculus which was considered to be normal in the first two locations and either normal or of minor importance in the latter two.

26. Pyknotic nuclei and albuminous degeneration were observed in the tubule cells of the kidneys stained with hematoxylin and triosin.

27. The kidney and liver changes with the occurrence of six cases of perosis in male chicks suggest that the rations may have been deficient in choline, even though the rations contained more choline than recommended by the National Research Council and also the theory that choline deficiency in chickens is not manifested by the

described liver and kidney changes.

28. The increased cellularity in the center of the capillary tufts in the glomeruli of chicken kidney, as compared with mammalian kidney, is believed to be a normal characteristic.

29. Hyalinized areas appearing in the medulla of the thymus near Hassall's corpuscles are believed to be normal for the chick and these areas may eventually become Hassall's corpuscles.

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